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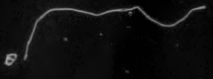

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THE GENUS PROROCENTRUM EHRENBERG. MORPHODYNAMICS, PROTOPLASMATIC STRUCTURES, AND TAXONOMY[1]

ADAM BURSA[2]

Abstract

The morphodynamics of *Prorocentrum* species have been studied comparatively in cultures and in plankton. Plankton populations show little morphological variation. In cultures a variety of forms, often similar to other species of *Prorocentrum* which have been described from distant seas, are found. Morphological variation in *Prorocentrum* species depends upon various life phases, individual features of the clones, and various types of the cysts. Formation of morphological aberrants is also affected by aging of cultures. All protoplasmatic structures, including the cell membrane, possess their own particular features, changing in the course of the life cycle. Since both physiological and morphodynamic metabolisms are inseparable in their activities, it is useful to complete taxonomic diagnoses with physiological observations. Five new species are described: *Prorocentrum cordiformis*, *Prorocentrum pomoideum*, *Prorocentrum pyrenoideum*, *Prorocentrum redfieldi*, and *Prorocentrum levantinoides*. Morphodynamics in *Prorocentrum micans* and *Prorocentrum scutellum* was also studied. Most of the observations were made in vivo. Specific morphological differences in form and structure of protoplasmatic organelles were demonstrated in each species concerned. An attempt was made also to base taxonomic diagnoses upon the apical tooth, trichocysts, and membrane structure.

Introduction

The genus *Prorocentrum* Ehrenb. comprises 31 species abundantly represented in the marine plankton of nearly all seas. Like most of the other dinoflagellates, Prorocentridae were studied mostly in the preserved state. In practice very incomplete data were obtained concerning general features of membrane form and structure. It is desirable therefore to know more about the morphological variation of the cell membrane and living protoplasmatic structures. Phytoplankton samples were collected over a great geographical area, and provide a sufficient basis for a comparative approach concerning the morphodynamics of various species. Cultures kindly given to me by Dr. M. Parke of Plymouth were most useful for experiments and study of all details found in this work. Previous observations of *P. micans* by Braarud and Rossavik (7) in cultures made my work easier, and to some extent are in agreement with my results.

These studies were carried out in the Mediterranean Sea from 1943 to 1945, in the English Channel in 1949 and 1950, in the North Atlantic in 1952.

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Contribution from the Fisheries Research Board of Canada.

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Phytoplankton collections taken by the *Calanus* Expeditions in 1953–1954 and 1956 were also examined, with no positive results as far as *Prorocentrum* species are concerned.

Materials and Methods

Materials used are as follows:

A. Living and preserved phytoplankton samples collected by the author on the coast of Israel, from June to September, 1943 and 1944.

B. Living and preserved samples collected by the vessel *Gammarus* of the Plymouth Marine Laboratory in August, 1939 and 1950, in the English Channel.

C. Single-celled and mixed cultures inoculated from Knap Buoy outside Plymouth, England, and kindly made available to the author by Dr. M. Parke. Clones *a*, *b*, *c* of *P. micans* were isolated in September 1949, and *Exuviella* sp. No. 18 was collected in June 1949.

D. Collections from Eel Pond and adjacent waters, Woods Hole, U.S.A., collected by the author in July and September 1952.

E. Bacterized cultures of *P. micans* obtained from Dr. L. Provasoli, Haskins Laboratories, N.Y. (originally sent to Dr. Provasoli by Dr. M. Parke in 1952).

F. Phytoplankton samples collected by the author in the Point Barrow area, Alaska, 1954.

G. Phytoplankton samples collected by the *Calanus* Expeditions in 1953 and 1954.

Neutral red and cresyl brilliant blue (0.1 and 0.3% in aqueous solution) were used for staining in vivo. A little drop of solution placed upon the cover slide was dried, then used to cover a drop of medium containing *Prorocentrum* culture. The gradual penetration of cresyl blue into protoplasm assures slow staining of organelles without destruction. Rapid preserving of *Prorocentrum* was obtained with boiling 10% sublimate and Lugols. Gram's iodine, Schweitzer solution, Marine Dafanos, Champy's fluid, and Janus green were used for staining mitochondria and trichocysts.

Geographical Distribution of *Prorocentrum micans* Ehrenb.

The earliest note of *P. micans* was published by Michaelis (43). It was drawn, mentioned, and described by Ehrenberg (23), Bütschli (14), Schütt (59), Kofoid and Swezy (37), Lindemann (39), Lebour (38), Stein (61), Braarud and Rossavik (7), Biecheler (10), Doflein (20), and other authors.

According to Braarud and Rossavik (7) it is reported from nearly all seas and limited in distribution to between 60° N. and 60° S. It is rare in pelagic, and common in neritic and brackish waters. As pointed out by Gaarder and Gran (25) it is one of the most important dinoflagellates in the Oslofjord plankton. Its maximum recorded by Braarud and Rossavik (7) is in late August and September. Optimum salinity lies between 15 and 20 parts per thousand according to Braarud (7). It is often dominant in the coastal water-blooms of California (Allen (1), Galtsoff (26)). It constitutes the main food of

oysters, according to Martin (42) and Schiller (56, 57). Orton (after Lebour (38)) says it is endobiotic in sponges. It was also squeezed from small sponges floating in Plymouth plankton in 1949 by the author (Fig. 48); whether symbiotic or accidental is unknown. In tracing the geographical distribution, it is necessary to compare this organism from various localities. As comparative morphotaxonomy based on original materials practically does not exist, it is possible to compare only descriptions and drawings from previous reports. Differences in shape and structure of *P. micans* have been noted. The drawing of Kofoid and Swezy (37, p. 81) reveals a perforated membrane with a prominent apical tooth, central nucleus, and large and small anterior pusules. Lebour's drawings (38, Pl. I, Fig. 5) show a spherical pusule at the apical tooth base and posterior nucleus. Schütt (59) and Gran and Braarud (30) show *Prorocentrum* possessing an antapical spine, not reported by others. Cytomorphological observations show other differences also. Martin (42) shows two large stigmas and segmented chromatophores. Biecheler (10) shows a large anterior pyrenoid and central nucleus. It is felt that these structural differences are of importance for taxonomic descriptions of *Prorocentrum* species. These features cannot be evaluated without completing observations on the morphological cycle of different species. It is generally accepted that dinoflagellates represent a typically cosmopolitan group. In the present state of our knowledge on the geographic distribution of species it is too early to draw such conclusions, since vast areas of marine waters are still not investigated. Discoveries of Osorio Tafall (45) and of the present study indicate many new species. Patrick (48), discussing a similar problem in geographic distribution of diatoms, quotes Cleve, who wrote that many diatoms are cosmopolitan, occurring in all parts of the world, but that there are, on the other hand, many species and genera which occur only in certain areas and climates. No *Prorocentrum* species occurred in the Arctic Ocean or in eastern Canadian waters during the present extensive investigations. The very rare Prorocentridae in these waters were represented only by *Exuviella* sp. and *Cenchridium* species.

Orientation in Prorocentridae

Prorocentridae occupy an isolated systematic position among other dinoflagellates. *Paleophalacroma* (Schiller (58)) is thought to represent a transitional link between various *Prorocentrum* species and Dinophysiales. The transverse furrow of *Paleophalacroma* is represented by only a narrow horizontal ridge, while the longitudinal furrow is very reduced and without marginal wings (Schiller (57, 58)).

There is some disagreement as to orientation in Prorocentridae. The flagellate end is regarded as anterior, the valves as left and right according to Bütschli (14). The valves are homologous with the epitheca and hypotheca of Peridiniales, the seam represents the girdle, according to Schütt (59, 60). It is much easier to consider the valve with the flagellar pores as being on the ventral side, and the opposite valve as dorsal. As far as the analogies and

homologies found among various genera of living dinoflagellates are concerned, it seems too early to discuss these features without knowledge of the fossil forms from which contemporary phylogenetic lines probably were developed.

Morphological Variation of *Prorocentrum micans* Ehrenb. in the Sea and in Cultures

Our knowledge of form variation, even in common species, is still far from satisfactory, owing to a paucity of descriptions and the fact that they are based mostly on preserved materials. Graham (28) demonstrated that the form and structure of dinoflagellates is still practically unexplored. The investigations of Braarud (3, 4, 5) constitute a step forward in our knowledge of taxonomy, physiology, and morphology. Little is known on autecology of species. Vigorous growth in culture conditions not existing in the sea shows high adaptability of many forms (36). Woloszynska (65) makes the Baltic biotope responsible for producing some characteristic Baltic forms. According to Paulsen (49, 50), the great variability among certain groups of the marine flagellates is mainly caused by the absence of the sexual phase in their reproduction. There is no doubt that both arguments are correct, but each may apply to only a certain group of species.

Experiments carried out by Braarud (3, 5), Braarud and Pappas (6), and Braarud and Rossavik (7) make it possible to distinguish three different types of morphological variation in dinoflagellates.

(*a*) *Goniaulax tamarensis* in two clones shows definite morphological difference in spite of the fact that the cultures were kept under the same conditions (Braarud (3, 4)).

(*b*) *Peridinium trochoideum* shows no form variation after 5 years in cultures in various salinities, temperatures, and nutrient conditions.

(*c*) *Prorocentrum micans*, a "good species in the sea", produces many aberrants in cultures (Braarud and Rossavik (7)).

The morphological variation in *P. micans* is shown in Figs. 1–22. Three clones of *P. micans* cultured in the Plymouth Laboratory were analyzed from the morphological point of view. Over 3000 cells from plankton were also examined, to obtain information on variations between cultures and the sea. Though many aberrants were recorded, only the more characteristic forms were drawn or described, and the intermediate forms were omitted. The analysis of such a supposedly simple organism as *Prorocentrum* reveals so many details that it is almost impossible to deal with them by morphobiometrical and statistical methods. However, it is possible to conclude that some clone possessed its own small morphological variation, expressed in the number of membrane pores and their features, and in the shape and structure of the apical tooth. Some rare aberrants were only once observed, or occurred only in one of the clones: for example, a *P. micans* which was strikingly similar to *P. hentscheli*, with spear-like apical tooth. *P. gibbosum* was also present in clone *b*. A similar aberrant occurred also in Braarud and Rossavik's cultures.

It is interesting from the taxonomic and geographical point of view that aberrants from cultures resembling *P. adriaticum* Schill., *P. arcuatum* Isel., *P. gibbosum*, and *P. hentscheli*, if found in the sea, would be identified as the above species.

Because at the present moment insufficient evidence has been obtained on *P. micans* species, and the nature of aberrant forms resembling other species, we name them *formas*, reviewed as below: (*a*) *P. micans* forma *typica* from plankton, shows slight variation (Figs. 1–3); (*b*) *P. micans* forma *subtypica* from cultures (Figs. 4–6); (*c*) *P. micans* aberrants, similar to species other than *P. micans* recorded in other distant seas (Figs. 11–22).

Aberrants similar to *Prorocentrum spheroideum* Schiller (56, 57), *P. ovalis* Rampi (53). *P. triangulatum* Martin (42), (Figs. 7–10), were common in all clones.

P. micans forma *delicatula* (Fig. 11).

P. micans without the apical tooth, with smooth wrinkled and bubbled cell membranes (Figs. 12–14).

P. micans deformed in various ways, probably as an effect of culture conditions (Figs. 15, 18, 22).

P. micans (clone *c*) with strong side constrictions (Fig. 19).

P. micans like *P. gibbosum* (clone *c*, Figs, 20, 21).

P. micans with apical tooth spear-like, similar to *P. hentscheli* recorded from the Atlantic (Schiller (56, 57)).

I conclude that there is parallelism in morphological variation in *Prorocentrum* cultures as between Braarud and Rossavik's and my observations.

The aberrants of *P. micans* previously described by Bergh (8), Bremen (11), and Hope (after Braarud) are very rare in plankton and their nature is still obscure. It is my view that aberrants in cultures originate as a result of senescence of crowded populations, and are probably affected by the nature of the medium. It may be possible to determine the nature of the various morphological aberrants by isolating them and growing them in clonal populations. However, in later sections it is shown that some morphological aberrants can develop from different forms of cysts, or during different phases of the life cycle.

Size Variation

The *Prorocentrum* species studied vary greatly in length and breadth, both in plankton and in culture. Three groups of species could be distinguished:

(*a*) small species like *P. cordiformis* sp. nov., Figs. 104–107;

(*b*) medium species like *P. pyrenoideum* sp. nov., Figs. 112–120;

(*c*) large species like *P. micans*.

The size of a given species can indicate its genotypic quality, though it varies also according to the individual and with the various phases of development. In *P. micans* and other species the length of cells varies between 20 and 80 μ. Within these limits intermediate cells occur. Individuals after and before cell division or during cyst formation vary highly. The length

and breadth of the motile cells are closely related to the form and shape of the cysts and their method of division. Spherical cysts, after the intramembranous cleavage, give rise to short broad motile cells, while long cysts produce narrow slim daughter cells, changing their shape in the course of later life, enlarging when cyst formation starts. It would be interesting to know the approximate size variation in relation to the vegetative and reproductive life phases associated with the morphological changes. The size variation of *P. micans* given below should also give information regarding the definite life phase in which the measurement was made; no such information is available because of technical difficulties. Dimensions are: from Lebour (38), length 36 to 48 μ, without apical tooth; from Schiller (56, 57, 58), length 36 to 48 μ; from Braarud and Rossavik (7), length 38 to 51 μ, breadth 17 to 38 μ; from present plankton samples, length 36 to 49 μ, breadth 27 to 39 μ; from present cultures, length 20 to 80 μ, breadth 15 to 35 μ.

The Membrane Pores in *Prorocentrum* Species

The membrane pores exist in many groups of plants. The complex pores in the desmids excrete mucus (Lütkemüller (40)). A diversity of pores in fossil desmids is described by Woloszynska (66). In most of the thecate dinoflagellates various types of membrane pores are found. In Prorocentridae membrane pores constitute typical generic features as demonstrated by Braarud (3) in the genus *Porella*.

In *Prorocentrum* the membrane pores vary in number and pattern according to the species. They change in individual cells according to their age and life phase. The membrane pores usually correspond to the number of trichocysts.

The membrane pores observed in plankton samples are well developed and mostly represented by tubular types of pores. In the cultures the tubular, transverse, and marginal pores almost disappear, though in a few individuals they remain. The distribution of pores in plankton and culture changes (Figs. 1–6). The membrane pores are classified, according to their shape and position within the valves, as:

f.p., flagellar pores, anterior and posterior (Figs. 25–27);
a.t.p., anterior tubular pores or simple pits (Figs. 1–3);
t.t.p., transverse tubular pores usually separated by central poroid field (Fig. 3);
p.ap., posterior tubular pores arranged in arches (Figs, 1, 2, 4, 6), transformed into simple pits in cultures;
p.p., posterior pores, a characteristic design for species with four to eight pores (Figs. 1–6);
m.p., marginal pores (Fig. 3);
s.p., suture pores at upper edges of the suture in *P. micans* and *P. redfieldi*;
t.f.p., tiny pits around flagellar tooth concavity (Fig. 25).

The transverse pores have larger outlets at the membrane surface, but smaller outlets at the surface in contact with the protoplasm. Their canals

are also often twisted. At the outlet of the pores a higher ring of membrane encircles the edge of some pores. The pores of ventral and dorsal valves build up symmetrical or asymmetrical patterns. The space between the pores is covered with punctuation, coarser towards the central poroid field, which consists of a system of small holes at the surface of the membrane but not perforating it. The tubular pores are usually turned towards the long cell axis with the exception of the pits. Thin-membraned individuals, examined after shedding their valves, showed the presence of the membrane pores. A peculiar pattern of membrane pores, consisting of two rows along the wide cell surface edges, occurred as an aberrant form in cultures (Fig. 6).

The short tubules were also observed in cultures of *P. micans* forma *delicatula* (Fig. 11). In the large spherical or subspherical species such as *P. scutellum*, *P. micans*, and *P. levantinoides* it is possible to identify them by a pore formula, though their arrangement varies. In narrow cells the number of pores and their structure is reduced, often to one or two rows of membrane pores. The spherical species like *P. pomoideum* are perforated with pores which bear specific features but are not arranged in a special pattern. This was observed by the author in *P. robustum* Tafall, *P. maximum*, *P. sigmoides* Tafall (45).

The hyaline, structureless valves of young cells stained with cresyl blue may reveal very delicate pores. Strong-membraned cells should therefore be chosen for studying membrane pore features.

The Apical Tooth in *Prorocentrum* Species

The apical tooth, an organ of unknown origin and purpose, represents a principal taxonomic criterion for distinguishing between the genera *Exuviella* and *Prorocentrum*. It may disappear in cultures (Barker (2)); also sometimes it is absent in plankton in *P. scutellum* (Martin (42)). According to the present author the apical tooth disappears in cultures of *P. micans*. In *P. pyrenoideum* and *P. levantinoides*, it could also be partially or completely reduced. Even in complete absence of this organ, the organisms could be identified by other typical membrane features. In *Desmocapsa*, *Porella*, and *Exuviella* small spines anteriorly located, as well as protoplasmic features, distinguish the genera.

Within *Prorocentrum* species the apical tooth is shown to be a highly variable and complex organ, spear-like in *P. hentscheli*, eminent and hollow in *P. dentatum*, sometimes containing protoplasm. Small *Prorocentrum* species have a simple apical tooth, reduced merely to a few spines located around the flagellar pores. The rudimentary apical tooth in *P. cordiformis* consists of two spines (Fig. 31; details concerning apical tooth structure are included in the figure explanations). In *P. pomoideum* sp. nov. the third spine is located between the flagellar pores (Fig. 32). In *P. redfieldi* (Fig. 34) the hyaline membrane stretches between two spines. A single spine and wing occur in *P. pyrenoideum* (Fig. 33). In *P. scutellum* it consists of a strong short spine, wide wing, and two or three small spines (Fig. 35). Well

developed in plankton, its form in *P. micans* (Figs. 36–41) is very complex and changing in details. It is undoubtedly different from *P. levantinoides* sp. nov. (Figs. 42 and 44). The forms and structures of the apical tooth in cultures are more variable than in nature.

Lebour (38) described the division of the apical tooth in *Prorocentrum* after the splitting of an isosceles organ into two even parts. Various forms of this organ were studied in cultures by the author. An individual *Prorocentrum* can develop a prominent apical tooth or it may be absent. Some cells possess only small spines resembling *Exuviella* species. As observed in cells produced after posterior division (Fig. 55), no apical tooth was formed or it was very rare. The apical tooth could be formed also inside the cysts. In cysts which elongate and become dorsoventrally flattened it is formed by exogenous droplets of ectoplasm, in the anterior part of the cell. In the motile cells from which naked protoplast escapes after the valves have opened it springs out immediately as a hyaline apical tooth (Fig. 85).

In spherical and subspherical cysts the apical tooth was formed during the course of their transverse constriction. The delicate stratification in the strong membrane cells was usually present. In rare cases the large apical tooth shows that there is a hollow capillary space which starts from the apical tooth base and ends at the top of the main spine. It is of phylogenetic interest that in clones of *Exuviella* species, grown from single cells, in rare cases the apical tooth occurred as a typical *Prorocentrum* apical tooth. These prove that both genera are nearly related (my observations).

Flagellar Pores and Closing Apparatus of the Valves

Two flagellar pores have been reported in most of the *Prorocentrum* species, and my observations agree. Single flagellar pores were recorded in *P. arcuatum*, *P. ovula*, and *P. nanum* by Schiller (57, 58). Two flagellar pores drawn by Biecheler (10) are of the same size, and are shaped like small boutonnieres as observed by the author. Two pores were observed by the author (Fig. 25); from the larger the longitudinal flagellum emerges, and from the smaller comes the transverse flagellum. The pores are also named "posterior" and "anterior". In aberrant individuals in cultures two pores perforating each valve have occurred (Figs. 26, 27). The flagellar pores are associated with the apical tooth and the complicated system of intravalvar structures (Figs. 36, 44, 46, 47). The diagnostic value of these structures is certain. It is convenient to call the perforated membrane valve by the flagellar pores the ventral valve, and the opposite one the dorsal valve. Dorsoventrality thus could be recognized by the presence of flagellar pores even in aberrant *Exuviella*-like forms without an apical tooth, or in other Prorocentridae which lack this organ.

Details of the closing apparatus of the valves were studied in *Porella* by Braarud (3). Owing to the small size of their structures the largest valves were selected for examination. Three modifications of the valvar closing

apparatus were observed by the present author: (*a*) indentations alternating with projections, interlocking the opposite edges of the valves (Fig. 23); (*b*) serrated edges of the sutures; (*c*) smooth edges of the sutures.

My experiments with hypertonic solutions of sea water show various efficiencies of closing forces against osmotic pressure in various species.

Some Features of the Cell Membrane

The viscous ectoplasmatic layer found under the membrane surface of the flagellated organisms produces various membrane structures by its power of coagulation. The evidence of this was obtained by discharging small ectoplasmatic droplets through the membrane pores of various *Prorocentrum* species. Such ectoplasmatic droplets immediately enlarge into large bubbles with solid membranes (Fig. 81, *e*). It was also recorded by the author in various thecate and naked dinoflagellates. The blue coloration of the ectoplasmatic bubbles of the rigid walls indicates cellulose. The cell membrane of the adult individuals of *Prorocentrum* species seems to be more complicated. Mangin (41) reported that the peridinean membrane is composed mainly of cellulose, pectins, and callose. The chemical composition of the membrane in *Ceratium* species changes, however, as the season advances, according to Pearsall (after Fritsch (24)). According to Braarud and Rossavik (7), the cell membrane of *Prorocentrum* shows cellulose reaction. According to the present writer, the staining capacity of *Prorocentrum* membranes is different in the young and adult phases. The single-layered young active individuals show even absorption of the vital cresyl blue, varying in the adult or aberrant specimens. Two- and three-layered cell membranes were composed of cellulose and an admixture of pectins. The inner membrane usually contained more pectins than the outer, indicating greater inclination for swelling. Secondary lamellation was discovered in many cases. Each *Prorocentrum* species concerned possessed some particular specific features. Three-layered membranes occurred in *P. scutellum* (Figs. 14, 18, 22).

Genus *Prorocentrum* Ehrenberg

Reproduction

Reproduction in Prorocentridae shows two peculiar features which probably represent phylogenetic relationships or merely convergences between dinoflagellates and diatoms. One is the method of longitudinal fission in *Prorocentrum*, in which the older valves are retained and two new inner valves are secreted during the separation of the daughter cells. The other is the intercalary growth of the suture which has a striking resemblance to the similar process in diatoms during the formation of auxospores. Reproduction in *Exuviella* recently studied by the author displays lines of development similar to those in *Prorocentrum*. The previous results and observations by Lebour (38), Schiller (56–58), and Braarud and Rossavik (7) are confirmed in this paper. The modifications in cell division observed are classified as below.

(*a*) Constriction starts at the antapex and is completed at the apex. Two even or uneven cells are formed after division (Figs. 49, 50).

(*b*) Constriction starts along the line of the suture and the final separation of daughter cells takes place in the middle (Braarud and Rossavik (7)).

(*c*) Constriction starts from the apex and is completed at the antapex (Fig. 53).

(*d*) Internal, oblique division of protoplast occurs inside the valves (Fig. 51).

(*e*) Lateral division takes place along the wide cell surface in *P. micans* cultures and in *P. pyrenoideum*.

(*f*) Antapical division (Fig. 55) was observed by the author in cultures; during such division three cells were formed (Fig. 54), sometimes attached to the substrate with mucus. Though it is thought that division of *Oxhyrris* (Hall (31)) follows the general pattern of dinoflagellates, conclusions cannot yet be made.

Cysts

Spherical and subspherical cysts in *Prorocentrum micans* were described by Braarud and Rossavik (7). Both types of cysts occurred only in the old cultures of the present author (Figs. 56, 57), but have never been observed in plankton by the author. Cyst development starts in motile individuals which usually enlarge their margins by the intercalary growth. There is no evidence to show why in some cells spherical cysts are formed while in others the cysts are subspherical and elongated. The pressure exerted by growing cysts opens the valves wide, as drawn by Braarud and Rossavik. According to the present author, two daughter cells may develop inside the cyst. The division of nucleus, pusules, and chromatophores starts almost simultaneously in most cells observed by the present author. Cyst membranes are formed from the inner layers of valves which in their early phases seem to be homogeneous. Some cysts (Fig. 52) have heterogeneous membranes (Fig. 58). Liberation of protoplast from the cysts (Figs. 56, 57) was induced by the author by temperature change. The cyst surface can be sculptured with fine dots or long streaks (Figs. 59 and 60), and can be colorless or brown. Cysts can enlarge in length and width. Thin-membraned cysts can produce two individuals after longitudinal or transverse cell division (Figs. 59, 60). Flagella are shot from the outside. Gradual polar rearrangement of nucleus and pusule position, and formation of apical tooth and membrane pores, are accomplished by tiny drops of ectoplasm leaking out. Some cysts were able to grow and divide (Figs. 60, 61, 62). Aberrant types of division are shown (Figs. 63, 64). In Fig. 63 the uneven cells are spherical in cross section, while in Fig. 64 they are dorsoventrally compressed.

Chromatophores

Holophytic flagellates contain leucoplasts, according to Schütt (after Schiller (56, 57)). Carotin, phylloxanthin, peridinin, and phycocyan have been extracted from holophytic forms. Strain (62) has demonstrated the presence of chlorophyll, diadinoxanthin, dinoxanthin, dinoxanthinin, neodinoxanthin, and peridinin. The principal carotinoid in *P. micans* is hydroxylated peridinin (Graham (29)).

A single chromatophore corresponding to the form of the valve is contained in each half of the cell membrane. It varies, according to species, from dark yellow to dark brown. The concentration of the chromatophore pigment depends on intensity of light and age of specimens. In young cells it is dark; in adult cells it is covered by reserve materials. The pigment of the chromatophore layer is separated from the cell membrane by ectoplasm. The surface of chromatophores kept in darkness shows fine mitochondria, stained with Schweitzer solution. They are rod-shaped and consist of cortex and inner fluid in which finer particles perform Brownian movement (Fig. 65). The above elements could be studied also at the surface of the pusule (Fig. 69). Long and short perforations in chromatophores appear during cell division (Figs. 65, 66) and are associated with formation of the trichocysts. In *P. pyrenoideum* (Fig. 67) trichocysts probably originate within regular protoplasmatic areas, observed during cell division. The fragmentation of chromatophores (Braarud and Rossavik (7), Martin (42)) takes place when large quantities of paramylum are grown in the protoplasm. Chromatophores in cysts were mostly disorganized.

Pyrenoids

The occurrence and structure of pyrenoids in *Prorocentrum* and other dinoflagellates have been little studied. They have been drawn in Desmocontae by Pascher (47), in *Heterocapsa* by Schütt (60), in *Peridinium* by Geitler (27), and in algae by Churda (17). Pyrenoids in the genus *Exuviella* constitute the characteristic feature; according to Thompson (63), in some cells they may disappear. Biecheler (10) studied pyrenoids in *Gymnodinium* and *Peridinium*, and created species based upon the presence of pyrenoids. In *P. micans* they are found in some specimens in cultures grown from a single cell; in others, however, they are absent. Biecheler described a large anterior pyrenoid in a *Prorocentrum* identified as *P. micans*. Such, however, was not noticed by the author in this species, either in plankton or in cultures, although several small pyrenoids, about 4–6 μ and from 8 to 28 in number, were observed embedded in chromatophore pigment or loose in protoplasm (Figs. 71–74). Pyrenoids in *P. micans* consist of spherical stroma containing within their margins tiny rodlets of starch, arranged peripherally around the center. The large pyrenoid located within the central part of the chromatophore was distinguished, in most cells, as a colorless body, but sometimes it was covered by chromatophore pigment in which no starch was evident (Figs. 75–78). Its division into two or four parts was also observed (Figs. 75–78).

Single pyrenoids on *P. cordiformis* sp. nov. and *P. pomoideum* sp. nov. possess large stroma. Their starch rodlets have an orange tint.

According to the present author some individuals of the species contain pyrenoids while others have no pyrenoids. It seems, therefore, risky to base taxonomic diagnoses on the absence or presence of pyrenoids alone.

Vital Colorations

According to Dangeard (19), in *Ceratium* a system of small vacuoles which stain with cresyl blue is dispersed over protoplasm. *Prorocentrum* species absorb vital stains differently probably according to capacity of the particular species and the life phase. As soon as a drop of medium containing *Prorocentrum* was covered, gradual absorption of cresyl blue was observed. It appeared first around the pores, the apical tooth wing, and the spines. The delicate blue shadows soon changed into the intensive deep blue. The thin-membraned cells showed homogeneous absorption of cresyl blue. The adult thick-membraned specimens showed more intensive color in the deeper layers. Very intensive blue occurred in aberrant forms, especially in the specimens with bubbles (Figs. 14, 16, 17, 18). This indicates also different properties of the middle membrane layer. The penetration of cresyl blue solution probably takes place only through the membrane pores and trichocysts, the latter slightly colored in the undischarged state. A few seconds after the pigment penetration began trichocysts were discharged inside or outside the cell (Fig. 84) and often were disintegrated. The vacuome elements absorbed cresyl blue later, showing varying degree of colors (Fig. 79). The strong coloration took place within areas of the disintegrated trichocysts, appearing as long dark tongues (Fig. 79). Threads like protoplasmatic structures attached to the membrane remained unstained, then rapidly stained. Thin-membraned cells were blown up, flagella were rejected, and pusules were pushed deep into the cell (Fig. 80).

The "Central Ring" and Pusules

Beneath the central part of the valves in some *Prorocentrum* species there is a structure named by the author the "central ring". It consists of fine amyloid bodies forming a regular spherical ring. This structure is shown in *P. micans*, *P. scutellum*, *P. levantinoides*, and *P. pyrenoideum* (Figs. 64, 79, 82, 133).

The pusules, still enigmatic organs in dinoflagellates, show complex structures in the pelagic forms. In Prorocentridae they are represented by a single pusule with a fine exit canal opening into the flagellar pore (Fig. 79). This organ is extremely sensitive to salinity–temperature changes. It detaches in the early phase of staining with cresyl brilliant blue (Fig. 80). In some *Prorocentrum* specimens two or three pusules were observed. In one case the exit canal of the pusule was fixed in the membrane pore. Pusules contain viscous fluid, not absorbing cresyl blue or neutral red. The delicate hyaline membrane is flexible and colloidal-like. It is covered by a layer of chromatophore pigment in which mitochondria-like bodies are densely located (Figs. 69, 70). Its presence in the motile cells seems to be associated in some way with flagellar movements which cease immediately after the discharge of the pusule. It occurs in all species of *Prorocentrum* concerned, except *P. redfieldi*, continuing still its movements after discharge of the pusule.

Trichocysts

Various types of trichocysts occur in holozoic and holophytic flagellates (Scherffel (55), Parke (46), Chadefaud (15)). Woloszynska (65) discharged trichocysts in *Gyrodinium fuscum*, Conrad (18) in *Chilodinium*. Bütschli (14) and Chatton (16) discovered "cnidocysts" in *Polykrikos* similar to those known in various Coelenterata. Kofoid and Swezy (37) record rhabdosomes in various genera of naked dinoflagellates. Bursa (13) studied trichocysts in *A. semilunatum*. Biecheler (10) has shown these organelles in dinoflagellates. Dragesco (21, 22) investigated trichocysts with the electron microscope. Trichocysts were recorded in all *Prorocentrum* species studied in this work. Trichocysts in *P. micans* and other species were seen well against the colorless pusule background. After treatment with acid (1.0 *N* HCl) trichocysts shrunk on the surface of protoplasm (Fig. 87). They look like long colorless rods in which alternating brighter and darker segments are clearly visible (Fig. 88). Temperature and salinity changes, as observed by the present author, produce external and internal trichocyst discharge or their disintegration into spherical bodies (Fig. 89). Similar effects could be obtained with various reagents such as Javel water, a boiling or cold solution of sublimate, acids, and cresyl blue. Rapid and complete trichocyst discharge in less than three seconds was produced with Javel water (Fig. 94). The length of the discharged trichocysts was more than 15 times greater than in the undischarged phase. The trichocysts discharged with 5% NaOH agglutinate and swell (Fig. 93). Treated with acid vapors they become stiff and break into many parallel needles (Fig. 90). For the first time axion and cortex in trichocysts were observed with front phase illumination. Schweitzer solution and Lugols stain selectively the axion and the cortex, the axion becoming dark violet or brown, the cortex in the beginning yellow or bright violet (Fig. 92). After a few seconds these heterogeneous structures disappear, and trichocysts become uniformly dark. Some discharged trichocysts turn into small droplets (Fig. 91).

It seems peculiar that fresh plankton samples of *Prorocentrum* often do not discharge trichocysts or only from few membrane pores. The trichocysts discharge was often complete when using the culture samples. The mass-discharged trichocysts formed an opalescent sediment found in the culture flask. This sediment contained, however, amorphous, broken, or swollen trichocysts. Since experiments show that penetration of sea water into the protoplasm takes place through the ends of trichocysts locking the membrane pores, trichocysts presumably act as filters in outward and inward diffusion, while the solid membrane seems to be osmotically indifferent. Trichocysts seem to be of no vital importance for life maintenance, since even specimens with completely discharged trichocysts can regenerate their motility again. Even motile *Prorocentrum* presumably in good metabolic conditions rarely contained complete sets of their trichocysts, while a great percentage of culture population possessed partially discharged trichocysts. The swelling of trichocysts or forming of nails or pegs locking the membrane pores (Fig. 86)

seems to be accidental and probably has no particular physiological meaning. It is finally concluded that trichocysts of the type concerned do not serve defense purposes of Prorocentridae.

Experiments with Hypotonic and Hypertonic Sea Water

The purpose of the experiments was to define the osmotic resistance of *P. micans* against hypotonic and hypertonic solutions of sea water, and other biological responses. The osmotic resistance in various groups of algae varies according to environmental conditions (Höfler (33, 34)). According to Biebl (9), deep-water algae have lower osmotic resistance than shallow-water algae; it is from 0.5 to 1.49. Algae exposed to the air have an osmotic resistance of 0.3 to 2.2 in the intertidal area. The limit for the deep-water Rhodophyceae is between 1.4 and 1.5. Higher concentrations become harmful to the organisms. Normal sea water or water 1 taken from 10 meters depth near Plymouth was used, gradual dilutions being obtained by mixing with ordinary tap water. The hypertonic solutions were obtained in a similar way by using water 2, a double concentration of normal sea water. A drop of culture medium containing living *P. micans* was placed upon the slide, then mixed with a drop of distilled water of the same size and observed immediately under the microscope. The results are given in Table I. The investigated organisms demonstrated great sensitivity and even transfer from the culture flask to the slide produced a short shock which rendered them motionless for about a minute. A second purpose was to determine lethal effects of hypotonic and hypertonic solutions. It was observed by the present author that advanced plasmolysis associated with nuclear displacement and trichocyst disintegration does not kill *Prorocentrum*, which may regenerate its structure and carry out all its activities again.

TABLE I

Experiments with hypertonic and hypotonic sea water

Experiment	Concentration	Results
No. 1, with hypertonic solutions	1.50	All cells dead, complete plasmolysis
	1.40	All cells dead, partial or complete plasmolysis
	1.35	All cells dead, partial plasmolysis
	1.30	After 3 to 5 minutes some cells recovered from the shock, trichocysts discharged, flagella rejected
	1.25	After 1 minute some cells motile again
	1.15	Most cells recovered and motile after short period
	1.10	No shock observed
No. 2, with hypotonic solutions	0.25	Rapid plasmolysis and protoplasm discharge
	0.30	All cells dead, cell membrane burst
	0.50	Mass discharge of trichocysts, all cells dead
	0.60	After 1 to 3 minutes some cells recovered
	0.70	After 23 minutes movements restarted in strong-membraned cells; thin-membraned cells burst
	0.80	No shock observed, all cells motile

No harmful effect is caused by the hypertonic solutions of concentration 1.30 and 1.25. The critical concentration associated with mass trichocyst discharge is 1.30. All lower concentrations produce rather small shock or none at all. In the hypotonic solutions of sea water mass discharge of trichocysts and strong plasmolysis occurred at 0.5 concentration. All cells were motionless or dead. A concentration of 0.6 is one from which some cells can recover after 1 to 3 minutes. In a concentration of 0.7 most cells become motile, though young thin-membraned cells disintegrate. No shock occurred at the concentration of 0.8.

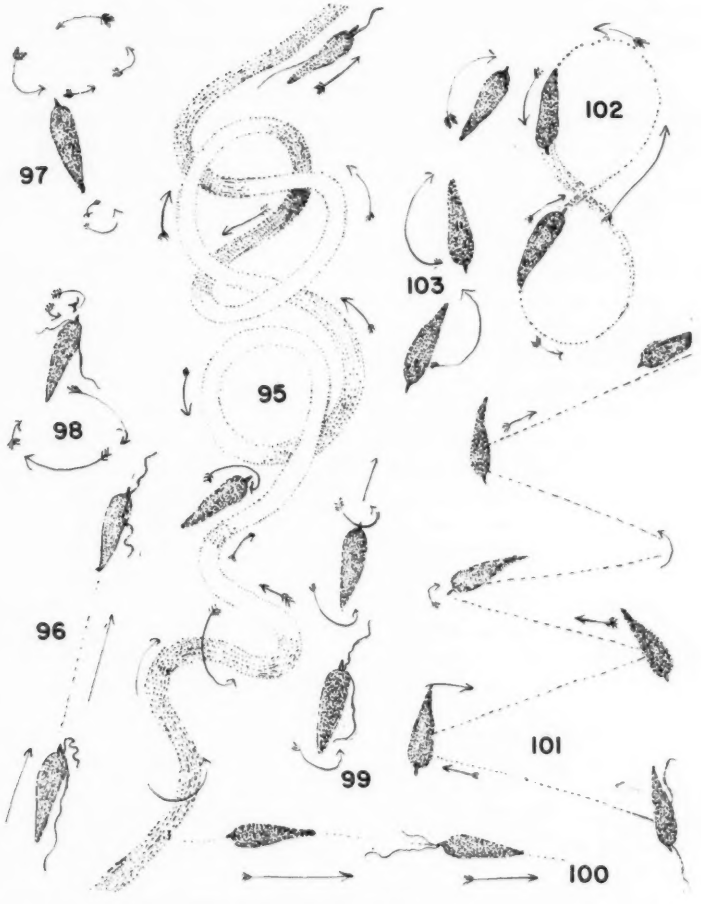
Movements in Prorocentrum Species

Flagellar movements in various genera vary specifically according to Peters (51). *Porella* produces a quick spiralling around an axis which passes through the cilia pole and the center of the cell (Braarud (3)). In *Exuviella compressa* one flagellum is directed forward and the other is whipped about, causing the cell to rotate on its long axis. At the same time the whipping flagellum trails and the cell glides along without revolutions (Thompson (63)). *Haplodinium* and *Prorocentrum* move in similar ways. The thread-like flagellum directed anteriorly pulls the cell forward, whilst the second flagellum swings transversely about causing rotation of the individuals (Fritsch (24)).

The above observations of flagellar movements mostly concern a single short phase of flagellate movement. However, more continuous observations of the movements show unexpected qualities in various species. Eight species of *Prorocentrum* studied by the author produced various types of flagellar movements. It seems almost impossible to describe all the modifications in each organism and the changing positions of the flagella and of the cell. Special attention was taken, therefore, to investigate various positions of the cell during movement. From observations on *Prorocentrum* genus, each species possesses its own type of movement, associated with its own morphodynamic features. Motile cells packed heavily with paramylum or strong-membraned aberrants produce different types of movements. In general, small species like *P. cordiformis* show much faster movements than the large *P. scutellum* or *P. micans*.

P. redfieldi was chosen as most convenient for observation. Eight types of movements occurred in this species, Figs. 97–103 indicating the details. More common kinds of movements are shown (Fig. 95), jet-like forward movements (Fig. 96), rotation of apex and antapex (Figs. 97 and 98), rotation through the short axis in a forward direction (Fig. 99), backward zigzagging (Fig. 100), tumbling (Fig. 103), and formation of the figure "8" when fixed with the posterior flagellum at the substrate. Some *Prorocentrum* possessed three flagella, as also reported by Ohno (44) in *Gymnodinium*.

In jet-like backward jumps (Fig. 100) both flagella were almost motionless. It is supposed therefore that some other kinetic forces act in this peculiar case. The anterior seems to be more active than the posterior flagellum. The anterior flagellum, though its position varies from anterior to lateral,



FIGS. 95–103. Movements of *P. redfieldi*. ×500.
FIG. 95. Common type of movement consists of spiralling, looping, combined with gyrating.
FIG. 96. Forward movements in straight lines.
FIG. 97. Rotation of apex in large circles, and of the antapex in small circles.
FIG. 98. Opposite to movements in Fig. 97.
FIG. 99. Forward movements in straight lines, rotation along short axis.
FIG. 100. Backward, discontinuous jumps in straight lines.
FIG. 101. Sudden zigzagging at various angles.
FIG. 102. A cell described a figure "8" while fixed with its posterior flagellum to the surface of the substrate.
FIG. 103. Forward tumbling.

works mostly in front of the cell and around the apex. It also performs vibrations while stretched forward. Its undulations, moving from the tip of the base, vary greatly from large waves of high amplitude to small waves of low amplitude. The posterior flagellum generally occupies a position beneath the cell and trails behind. It seems less flexible than the anterior flagellum though it acts as a steering mechanism in forward and backward movements. Four motile cells of *P. micans* in division performed well-synchronized flagellar actions. No true observations on the flagellar movements can be made under a low power microscope. It is necessary to change objectives from low power to oil immersion very quickly to catch the unexpected jumping, zigzagging, or tumbling.

Recent investigations reveal high structural differentiation of flagella as shown by the electron microscope (Brown (12), Pitelka (52), Houwink (35), and Vlk (64)). The flagellar apparatus in *Euglena* responds to the stigma stimulated by light intensity and quality. It is concluded that hydrodynamics of flagellates and causes of peculiar movements are still unknown. They are, however, of great efficiency if compared with the small size of these organisms performing diurnal vertical migrations within several meters depth as shown by Hasle (32).

Taxonomy

Prorocentrum **cordiformis** sp. nov. (Figs. 104–107)

Heart-shaped cells, wide at anterior end, narrow at posterior end. Short, simple spine located near the edge of the flagellar pore, an additional smaller spine opposite to it. Spherical and subspherical brown-yellow chromatophores, some of them anastomosed. Two uneven flagella emerge from two distinct flagellar pores. Movement fast, spiralling with frequent tumbling. Cell membrane smooth and very thin, shows no trace of membrane pores, flexible, easily deformed. Three to six trichocysts, rod-shaped, anteriorly located. One or two spherical pyrenoids loose in the protoplasm, occupying a central position in the cell. No suture between the valves was distinguishable.

Dimensions.—Length from 6 to 12 μ; breadth from 5 to 9 μ; height of the apical spine, 1.5 to 2 μ.

Occurrence.—Isolated as *Exuviella* sp. No. 18 by Dr. M. Parke, Plymouth, Knap Buoy, June 1949, at a depth of 4 fathoms.

Affinities.—The organism is undoubtedly distinct from any other known species by its small size as well as by other morphological features. It occupies an intermediate taxonomic position between *Prorocentrum* and *Exuviella*. Since it has two uneven anterior spines it seems to be more closely related to *Prorocentrum*.

Prorocentrum **pomoideum** sp. nov. (Figs. 108–111)

Apple-shaped cells in surface lateral view, often with a posterior constriction (Fig. 111). Flattened dorsoventrally, with a well-marked suture. Cell membrane perforated with numerous small membrane pores. Some individuals are covered with tiny spines; other are smooth. Two flagellar

pores, a larger anterior and a smaller posterior one. Two uneven flagella cause fast spiral movement with frequent rotation alternating with tumbling. Main apical tooth without a wing, located at the edge of anterior flagellar pore. Second spine located within the isthmus between the flagellar pores. The third spine shortest, located at the edge of the flagellar cavity. The flagellar pores are embedded in a small shallow flagellar depression forming a separate membrane plate which is fused with the valve. Single large yellow chromatophores are present in each valve, with two anterior perforations where the pusule is located. The posterior chromatophore perforation is found within the nuclear area. In some cells the chromatophore plate is folded around the nucleus. Spherical paramylum grains are present, often bright orange, especially within the posterior part of the cell. Subspherical nucleus close to antapex.

Four to 11 rod trichocysts are present, not attached to the membrane pores, located mostly within the anterior end of the protoplasm. Pusule spherical with a fine exit canal through the anterior flagellar pore. Trichocysts were discharged with Lugol solution, have a small droplet at the top.

Dimensions.—Length from 9 to 20 μ; breadth from 11 to 16 μ.

Occurrence.—Isolated and cultured by Dr. M. Parke, from Yamar Estuary, June 1950.

Affinities.—It seems to be related to *P. obstusum* Schiller (57, 58) from the Mediterranean, which is a larger species with a single apical tooth.

Prorocentrum **pyrenoideum** sp. nov. (Figs. 112–120)

Cells long, fusiform, dorsoventrally flattened, narrow in sagittal view, wide in side view. Posteriorly pointed, anteriorly rounded, with a short apical tooth 1.5 to 3 μ high, placed near the margin of the ventral valve. The apical tooth has a hyaline wing, with a thin main spine. Sometimes an additional little spine is observed opposite the main spine, fused to the wing membrane (Fig. 34). Two uneven flagellar pores can be observed in front view. In side view they are seen as wider and narrower tubules. Flagellar movements are faster than in *P. micans* (Figs. 79–100). The membrane pores within the sutures are mostly simple pits or narrow tubules. In rare cases tiny posterior membrane pores were also observed. Two transverse strips of membrane interlock within the flagellar cavities of both valves (Fig. 113). In aberrant forms a single large funnel-like pore, resembling the membrane pores in *Porella*, was observed in the middle of the valves.

Chromatophores yellow-green, one in each valve, with pusular perforations. One or two large pyrenoids within the center of each chromatophore, in some cells also laterally located.

Posterio-anterior cell division often observed. Some cells with large borders increased by intercalary growth, often square in sagittal view. Cysts spherical. Paramylum as small rodlets or spherical grains. Nucleus subspherical, posterior. *Exuviella*-like cells often observed.

Dimensions.—Length, according to M. Parke, from 10 to 14 μ; length, according to the author, from 8 to 24 μ; breadth, according to the author, from 5 to 13 μ; height of the apical tooth, 3 μ.

Occurrence.—Isolated by Dr. M. Parke, Plymouth, Knap Buoy, June 1949, at a depth of 4 fathoms.

Affinities.—*P. pyrenoideum* shows some affinities to *P. triestinum* Schiller. It is known to the author from his own observations. *P. pyrenoideum* could be distinguished from *P. triestinum* by its large pyrenoid. The large, almost cubic paramylum grains found in *P. triestinum*, also figured by Schiller (56), are thought to distinguish it from other species. Other features characteristic for *P. pyrenoideum* are the long trichocysts located in various positions in the protoplasm (Fig. 118). After discharge they look like long thin needles with a tiny droplet at the top (Fig. 120). The most outstanding structures of *P. pyrenoideum* are long antapical tubular membrane pores (Fig. 118) found only in this species. *P. pyrenoideum* belongs to the medium-size species. It is clearly distinct from any other *Prorocentrum* species described by its *antapical tubular pores*, as well as by other structures. Its closest relative seems to be *P. triestinum* (Fig. 117).

Prorocentrum **redfieldi** sp. nov. (Figs. 121–124)

Cells long and slender, comma-shaped, oval in cross section, rounded anteriorly with a hyaline apical tooth in which the main spine and hyaline wing membrane are fused together, along with the second smaller spine (Fig. 34). Anterior and posterior membrane pores within the shallow depression at the base of the apical tooth. Two uneven flagella with rather changeable movements (Figs. 97–100). Both valves slightly convex, covered by simple pits scattered over the membrane surface with no definite distribution. In some cells the valves are perforated all around their margins. Short tubular pores were observed in only a few individuals. The general morphological features of some cells do not differ much whether seen in sagittal or ventral view.

A single yellow-green chromatophore plate in each valve. Small paramylum grains mostly in the anterior part of the cell, staining blue with Lugol solution. Two regular spherical trichocysts extending between the chromatophores. Pusule reservoir with a fine exit canal within the flagellar pore.

Dimensions.—Length from 18 to 32 μ; breadth from 6 to 10 μ; height of the apical tooth, 3 to 5 μ.

Occurrence.—Eel Pond plankton and in coastal plankton of Woods Hole, Massachusetts, from July to September, 1952. Mostly found in Tintinnidae, which select it as food. Neritic-brackish.

Affinities.—It seems to be closely related to *P. triestinum* and *P. schilleri.*

Prorocentrum **levantinoides** sp. nov. (Figs. 125–127)

Cell shaped like an almond kernel, broad at the anterior end. Strong valves with a prominent apical tooth (Figs. 42–44, 125), unlike *P. micans.* Membrane pores vary in different individuals (Figs. 125, 126), with well-developed marginal pores. Large poroids present in the center of both valves, or in some cells covering the whole valvar surface (Fig. 47). Two smooth membrane surface areas: near the apical tooth and at the edge of the posterior end of the cell. Two brown-yellow chromatophores, perforated by the

pusule reservoir which leads into a flagellar pore. The rod-shaped tricho cysts distributed according to the pattern of the membrane pores. Numerous paramylum grains dispersed within the protoplasm. A large "central ring" in the center of the chromatophore. Nucleus posterior.

Dimensions.—Length from 15 to 60 μ; breadth from 18 to 49 μ; height of the apical tooth from 8 to 12 μ.

Occurrence.—Coast of Israel, Tel-Aviv, Jaffa, from June to December, 1944.

Affinities.—It is closely related to *P. micans*, but distinct from it in respect to the apical tooth, membrane pore pattern, and trichocysts. There is a tendency in some cells toward reduction of the membrane pores.

Prorocentrum scutellum Schröder (Figs. 129–134)

Cells oval, laterally compressed, rounded posteriorly or in some cases pointed. The apical tooth short, consisting of a wide hyaline wing attached to the solid spine. The flagellar cavity shallow, one or two small additional spines found opposite the apical tooth. Woods Hole specimens compared with Martin's (42) descriptions and figures show identical features. The pattern of the membrane pores in *P. scutellum* has its own characteristic organization, varying according to the individual specimens. An asymmetrical distribution of the membrane pores within the valves occurred in most of the cells. In a few cases regular distribution of membrane pores was observed. Schiller's (58) drawings compared with the author's show great similarity between Mediterranean and Atlantic forms. The distinguishing three-layered cell membrane was often found in this species. In some cells the edge of the cell membrane is thin, in others wide. In sagittal view the cell is a lens-shaped structure with a well-developed apical tooth. The shape and distribution of trichocysts are specific for this species. Two of them are longer, and reach the center of the cell close to the "paramylum ring". Pusules larger than in *P. micans*, with a fine exit canal leading to a flagellar pore. Movements slow. A large nucleus close to the center of the cell changes to a central anterior or central posterior position. After shedding the flagella, under unfavorable conditions, the protoplasm shrinks, its innermost membrane swells, and it turns into a resting phase which resembles palmella.

Dimensions.—Length from 36 to 57 μ (Lebour (38)).

Occurrence.—It has been reported from the Adriatic Sea, the Bosphorus, the Mediterranean, the English Channel, the Ligurian Sea, the North Sea, and on both sides of the North Atlantic. It seems to be a cosmopolitan species. *P. robustum* Tafall (45) shows some close similarities in the morphology of its outlines. These similarities concern to some degree the structure of the apical tooth. It is certain, however, that the general patterns of the membrane pores in *P. scutellum* and *P. robustum* are different. It should be taken into account, however, that structural details in the former species were not studied. The chromatophore form in *P. scutellum* shows entirely different features from those found in the other *Prorocentrum* species mentioned in this paper. They have many perforations perpendicular to the

edges of the cell membrane occupied by trichocysts (Fig. 133). Some of the perforations divide the chromatophore plate into single lobes, others into secondary and tertiary lobes.

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EXPLANATION OF FIGS. 1–94, 104–134

FIG. 1. *Prorocentrum micans* f. *typica*, laid on the dorsal valve, ×1200, from Plymouth plankton. *a.t.p.*, anterior tubular pores; *t.t.p.*, transversal tubular pores; *a.p.*, arch pores; *p.p.*, posterior pores.

FIG. 2. Ventral valve from interior. ×1200. *s.p.*, simple pits; *p.f.*, poroid field; *p.p.*, posterior pores.

FIG. 3. Dorsal surface of the valve. ×1200. *m.p.*, marginal pores; *p.f.* poroid field.

FIGS. 4–6. *P. micans* f. *subtypica*. ×1200. Clones *a*, *b*, *c*; *s.p.*, simple pits; *f.p.*, flagellar pores.

FIGS. 7–10. Aberrants from the clones *a,b,c*, (×900) resembling *P. ovalis* Rampi (53), *P. spheroideum* Schill. (59), *P. triangulatum* Martin (42).

FIG. 11. *P. micans* f. *delicatula*. Dorsal valve with tubular pores. ×900.

FIG. 12. *P. micans* (*Exuviella*-like) with smooth membrane. ×900.

FIG. 13. *P. micans*. Wrinkled membrane, from inside. ×900.

FIG. 14. *P. micans*, bubbled membrane. ×900.

FIG. 15. *P. micans*, strong-membraned forms. ×900.

FIG. 16. *P. micans*. Aberrant with ingrowth. ×900.

FIG. 17. *P. micans*. Aberrant with outgrowth containing protoplasm. ×900.

FIG. 18. *P. micans*. Aberrant in which three membrane layers are distinguished after vital staining. ×900.

FIGS. 19–21. *P. micans* f. *gibbosum*, in various positions. ×900.

FIG. 22. *P. micans*. Aberrant form. ×900.

FIG. 23. *P. micans*. Closing mechanism of the suture covered with interlocking indentations (*i.i.*). Empty valves in side view. ×1500.

FIG. 24. *P. micans*. Empty valves in side view showing intercalary growth of the borders. *r.*, transverse membrane ribs. ×1200.

FIG. 25. *P. micans*. Front view of the apical tooth. *a.f.p.*, anterior flagellar pore; *t.f.p.*, tiny pits surrounding the apical tooth; *f.a.t.p.*, forked apical tooth part; *a.t.*, apical tooth. ×1200.

FIG. 26. *Exuviella*-like cells of *P. micans* shows flagellar pores, *f.p.* ×1200.

FIG. 27. *Exuviella*-like cell in side view with elongated flagellar pores. ×1200.

FIG. 28. *P. micans*. Observed from inside showing two-layered valve. ×1200.

FIG. 29. *P. micans*. Second inner membrane detached and forming cyst. ×1200.

FIG. 30. *P. scutellum*. Three-layered membrane observed from inside. ×1200.

FIG. 31. *P. cordiformis*. *a.t.*, apical tooth; *s.s.*, small spine. ×2500.

FIG. 32. *P. pomoideum*. *a.t.*, apical tooth; *i.s.*, isthmus spine; *s.s.*, small spine. ×2500.

FIG. 33. *P. pyrenoideum*. Parts of the apical tooth. *w.*, wing; *a.t.s.*, apical tooth spine. ×2500.

FIG. 34. *P. redfieldi*. *a.t.s.*, apical tooth spine; *w.*, wings; *s.s.*, small spine. ×2500.

FIG. 35. *P. scutellum*. *a.t.s.*, apical tooth spine; *w.*, wing. ×2500.

FIG. 36. *P. micans*. Laid upon ventral valve. *a.t.s.*, apical tooth spine; *w.*, wing; *i.s.*, isthmus spine; *b.s.*, two base spines; *a.f.p.*, anterior flagellar pore; *p.f.p.*, posterior flagellar pore; *e.s.*, edge spine. ×2500.

FIG. 37. *P. micans*. Focussed in middle depth. *t.l.w.s.*, transverse low wing support; *f.c.*, flagellar cavity; *a.t.s.*, apical tooth spine. ×2500.

FIG. 38. *P. micans*. *a.t.s.*, apical tooth spine; *u.w.s.*, upper wing support; *f.c.*, flagellar cavity; *e.s.*, edge spine; focussed at the bottom. ×2500.

FIG. 39. *P. micans*. Upon the dorsal valve is shown the deep edge of the flagellar cavity formed by the extended apical tooth spine. *u.w.s.*, upper wing support; *e.s.*, edge spines. ×2500.

FIG. 40. *P. micans.* *l.w.s.*, lower wing support; *c.*, five deep carvings at the bottom spine, stratification of the wing membrane; *a.f.p.* and *p.f.p.*, anterior and posterior flagellar pores. ×2500.

FIG. 41. *P. micans.* Ventral valve after removal of the apical tooth, showing the interior; *t.s.*, transverse membrane strips, a part of the locking apparatus. ×2500.

FIG. 42. *P. levantinoides.* *a.t.*, apical tooth spine; *w.*, wing; *f.c.*, flagellar cavity, intermediate position between lateral and sagittal surfaces. ×2500.

FIG. 43. Position reversed from Fig. 42. *e.s.*, edge spines. ×2500.

FIG. 44. *P. levantinoides.* Sagittal view. ×2500.

FIG. 45. *P. levantinoides.* Apical tooth and flagellar cavity, surrounded by a ring of simple pits. ×2500.

FIG. 46. *P. levantinoides.* Apical tooth from front view, and the structure of the membrane in the flagellar cavity. ×2500.

FIG. 47. *P. levantinoides.* Interior of the valve showing apical spine and wing, and the structure of the flagellar cavity. Dotted points poroids. ×2500.

FIG. 48. *P. micans.* Squeezed from the small sponge found in the plankton sample from Plymouth, England. ×250.

FIG. 49. *P. micans.* Early peripherical constrictions in cell division. ×1200.

FIG. 50. *P. micans.* Advanced antapical division. ×1200.

FIG. 51. *P. micans.* Oblique division of the protoplast. ×1000.

FIG. 52. *P. micans.* Individual in which the inner membrane layer is detached from the outer. ×1200.

FIG. 53. *P. micans.* Antapical cell division in its initial phase. ×1200.

FIG. 54. *P. micans.* Agglutinated three daughter cells in antapical division. ×1200.

FIG. 55. *Exuviella*-like cells after completed division. ×1200.

FIG. 56. *P. micans.* Excystment of protoplast produced by transfer from refrigerator to room temperature. *n.*, nucleus; *v.*, vacuole; *ch.*, chromatophore. ×1200.

FIG. 57. *P. micans.* Two daughter cells in the subspherical cysts. *p.*, pusule; *n.*, nucleus. ×1200.

FIG. 58. *P. micans.* Transverse constriction in a cyst with two-layered membrane. ×1200.

FIG. 59. *P. micans.* Initial phase of transverse cyst division. Brown sculptured cyst surface. ×1200.

FIG. 60. *P. micans.* Motile cyst-like cells; advanced division. ×1200.

FIG. 61. *P. micans.* Two daughter cells derived from spherical cyst. *n.*, nucleus. ×1200.

FIG. 62. *P. micans.* Advanced cyst division in front view. ×1200.

FIG. 63. *P. micans.* Aberrant cells before separation. ×1200.

FIG. 64. *P. micans.* Final phase of cell division shows: *p.*, pusules; *a.t.*, apical tooth formation after division. ×1200.

FIG. 65. *P. micans.* Perforated chromatophore in ventral view. ×1200.

FIG. 66. *P. micans.* Chromatophores segmented in sagittal view. ×1200.

FIG. 67. *P. pyrenoideum.* Regular protoplasmatic territories of chromatophore surface in dividing specimens. ×1200.

FIG. 68. *P. pyrenoideum.* Two chromatophores of a single cell in side view; notice the pyrenoids. ×1200.

FIG. 69. *P. pyrenoideum.* Part of the pusule and chondriosome-like bodies. Chromatophore in the background. ×2500.

FIG. 70. *P. pyrenoideum.* Surface of chromatophore with a mitochondria from which paramylum initiates. ×2500.

FIGS. 71–74. *P. micans.* Various forms of pyrenoids found in unialgal cultures. ×2500.

FIGS. 75–78. *P. purenoideum.* Division of pyrenoids. ×2500.

FIG. 79. *P. micans.* Side view; initial phase of cresyl blue coloration. *v.*, vacuome; *p.*, pusule; *d.t.*, disintegrated trichocysts; *p.g.*, paramylum grains; *p.r.*, paramylum ring; *n.*, nucleus. ×1200.

FIG. 80. *P. micans.* Stained with cresyl blue in sagittal view. *b.e.*, bubbles of ectoplasm discharged through the membrane pores; *m.i.*, bubbles of ectoplasm containing metachromatic elements. ×1200.

FIG. 81. *P. micans.* Partial plasmoptysis produced with hypertonic NaCl 1.30; *e.b.*, ectoplasmatic bubbles (posterior), containing trichocysts, chromatophore pigment, or empty; *t.*, containing only trichocysts. ×1200.

FIG. 82. *P. micans.* Radial arrangement of trichocysts around the "central ring"; *d.t.*, disintegrated rod trichocyst (before experiment). ×1200.

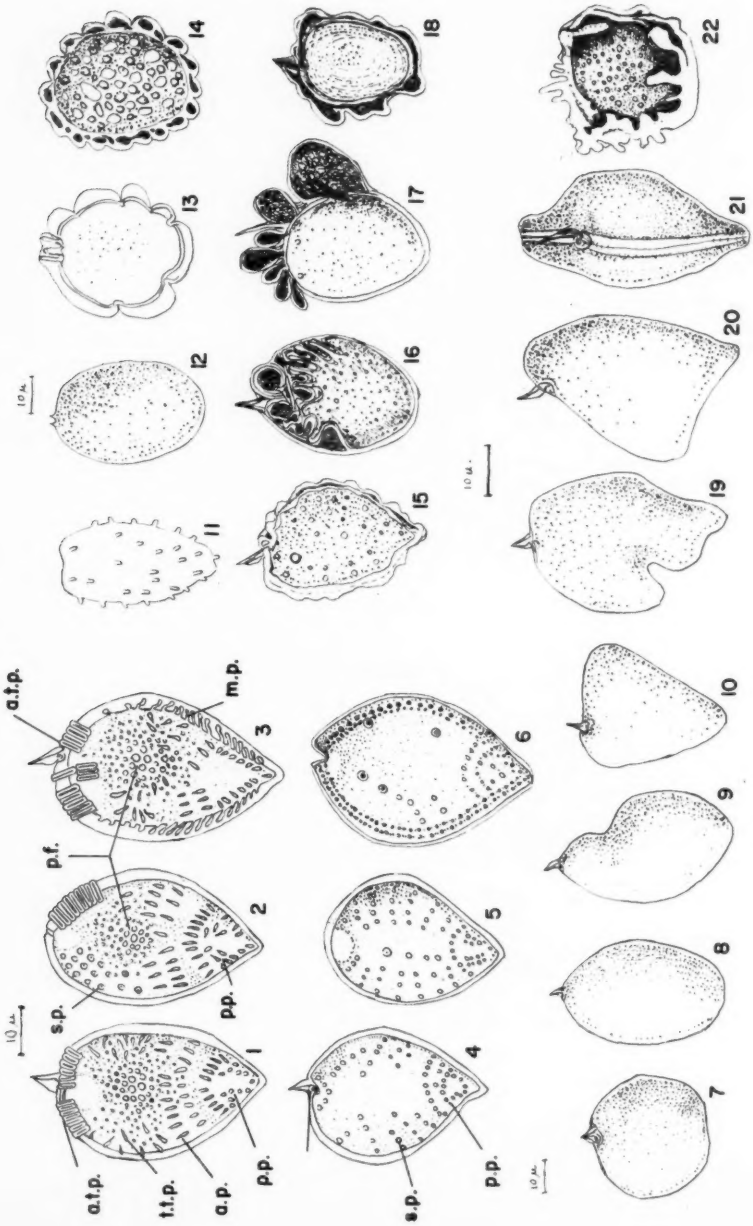
FIG. 83. *P. micans.* (Figs. 82–84, same specimen after a few seconds); after being treated with NaCl solution, trichocysts remained unchanged while protoplasm shrank. ×1200.

FIG. 84. *P. micans.* Destruction of trichoycsts, *d.t.*, into spherical bodies. ×1200.

FIG. 85. *P. micans.* Motile cell after rejection of both valves. ×500.
FIG. 86. *P. micans.* Plasmolyzed protoplasm, pegged membrane pores, with swollen trichocyst, *n.t.* ×1200.
FIG. 87. *P. micans.* Surface location of trichocysts is demonstrated by rapid shrinkage of protoplasm after treatment with acetic acid. ×1200.
FIG. 88. *P. micans.* Trichocysts' structure as observed in vivo. ×2500.
FIG. 89. *P. micans.* Trichocysts after disintegration, stained with Lugol solution and showing bright cortex and dark center. ×2500.
FIG. 90. *P. micans.* Discharged trichocysts, show dark axion and bright cortex, split lengthwise. ×2500.
FIG. 91. Discharged trichocyst turned into fluid droplets. ×2500.
FIG. 92. After treatment with Lugol solution the axion shrunk and broke into dark fragments, while sheath remained unaffected. ×2500.
FIG. 93. Swollen trichocysts after treatment with NaOH. ×2500.
FIG. 94. *P. micans.* Complete discharge of trichocysts, with Javel water. ×1200.

FIGS. 104–107. *P. cordiformis.* Four different individuals, showing pyrenoids, pusules, trichocysts, and chromatophores. ×1200.
FIGS. 108–111. *P. pomoideum.* Fig. 109 in sagittal view; others in ventral view. Trichocysts, membrane pores, pusules, and nuclei. ×1200.
FIG. 112. *P. pyrenoideum.* In sagittal view. ×1200.
FIG. 113. *P. pyrenoideum.* An empty ventral valve seen from inside. ×1200.
FIG. 114. *P. pyrenoideum.* *Exuviella*-like specimen in sagittal view. ×1200.
FIG. 115. *P. pyrenoideum.* Megacyst in sagittal view. ×1200.
FIG. 116. *P. pyrenoideum.* Cyst. ×1200.
FIG. 117. *P. triestinum.* Containing great characteristic paramylum grains. ×1200.
FIG. 118. *P. pyrenoideum.* Typical posterior tubular pores. ×2500.
FIG. 119. *P. pyrenoideum.* Trichocysts' location and pyrenoid. ×1200.
FIG. 120. *P. pyrenoideum.* Trichocysts' discharge. ×1200.
FIG. 121. *P. redfieldi.* Observed in vivo. ×1200.
FIG. 122. *P. redfieldi.* Empty valves. ×2500.
FIG. 123. *P. redfieldi.* In sagittal view. ×2500.
FIG. 124. *P. redfieldi.* Ventral valve, its marginal and posterior membrane pores. ×2500.
FIG. 125. *P. levantinoides.* Laid on the ventral valve. ×1200.
FIG. 106. *P. levantinoides.* An empty dorsal valve. ×1200.
FIG. 127. *P. levantinoides.* A small individual. ×1200.
FIG. 128. *Prorocentrum* sp. with undulated membrane surface. ×1200.
FIG. 129. *P. scutellum.* Ventral valve and its structure (alive), from Woods Hole. ×1200.
FIG. 130. *P. scutellum.* An empty dorsal valve. ×1200.
FIG. 131. *P. scutellum.* Ventral valve shows its membrane pores. ×1200.
FIG. 132. *P. scutellum.* In sagittal view. ×1200.
FIG. 133. *P. scutellum.* Chromatophore structure. ×1200.
FIG. 134. *P. scutellum.* Distribution of trichocysts, observed in vivo. ×1200.

NOTE: Figs 1–94, 104–134 follow.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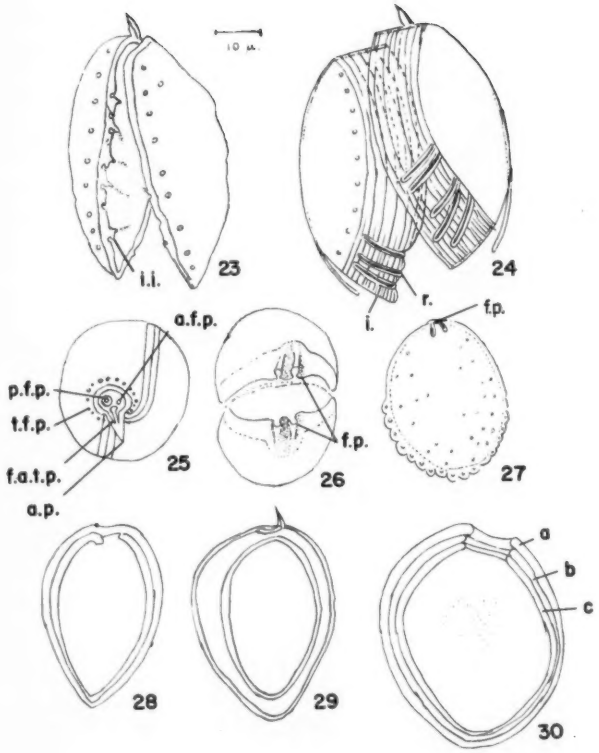
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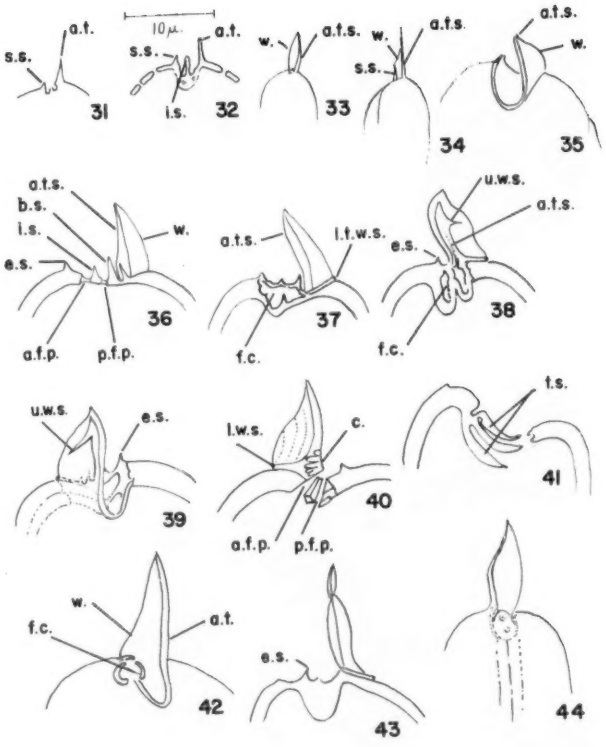
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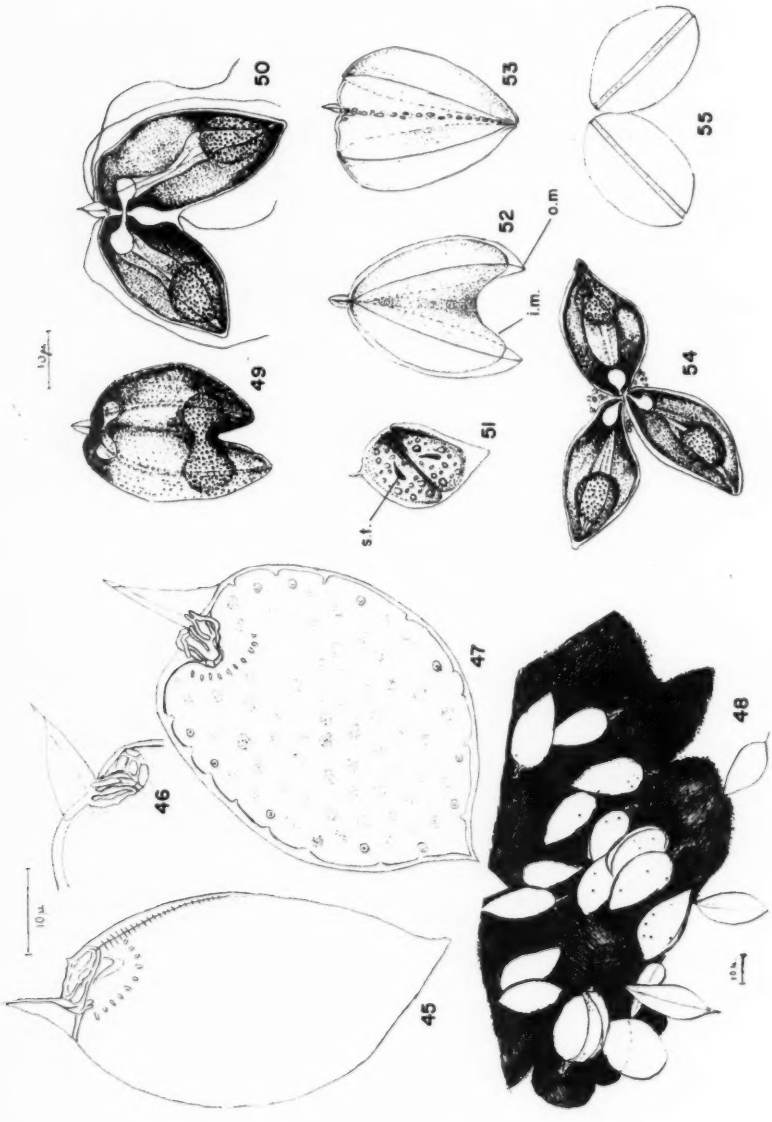
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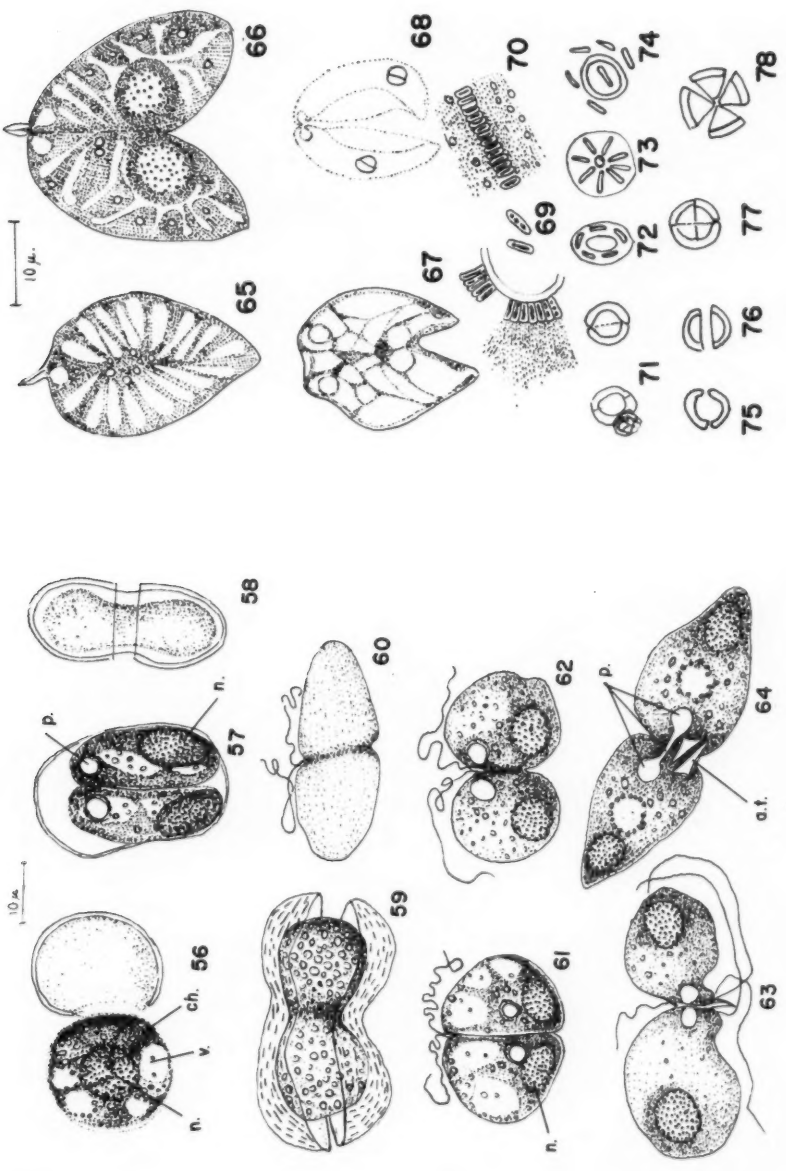
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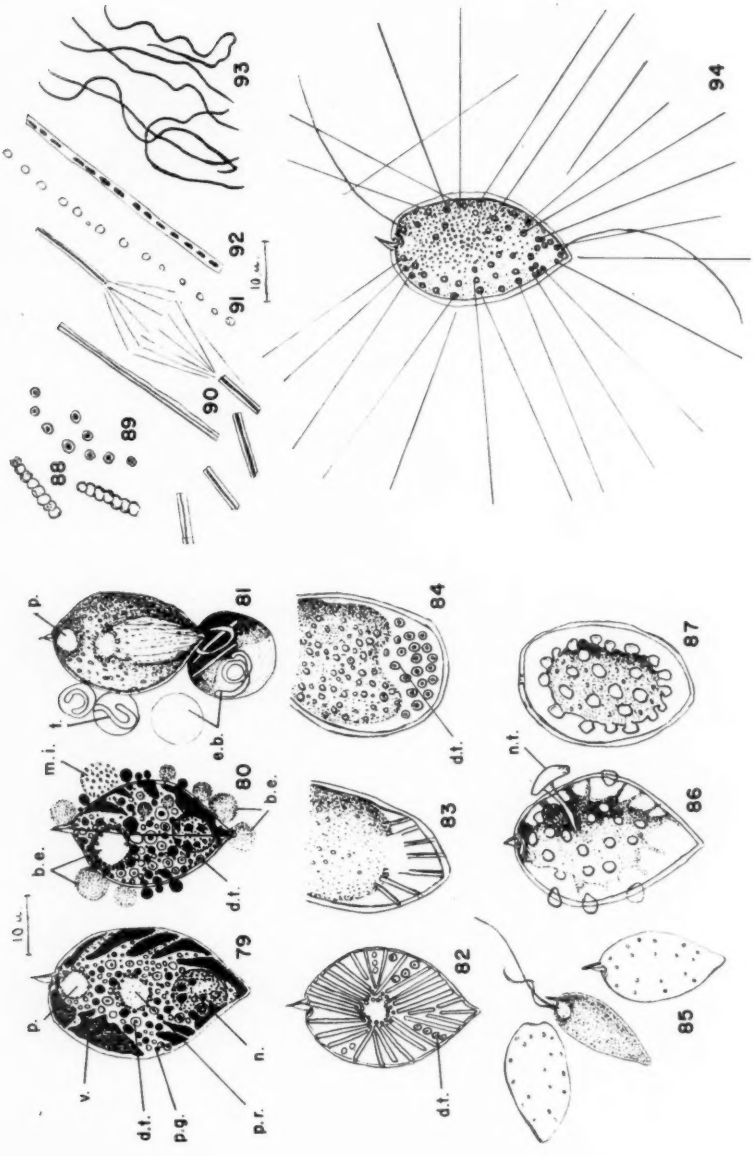
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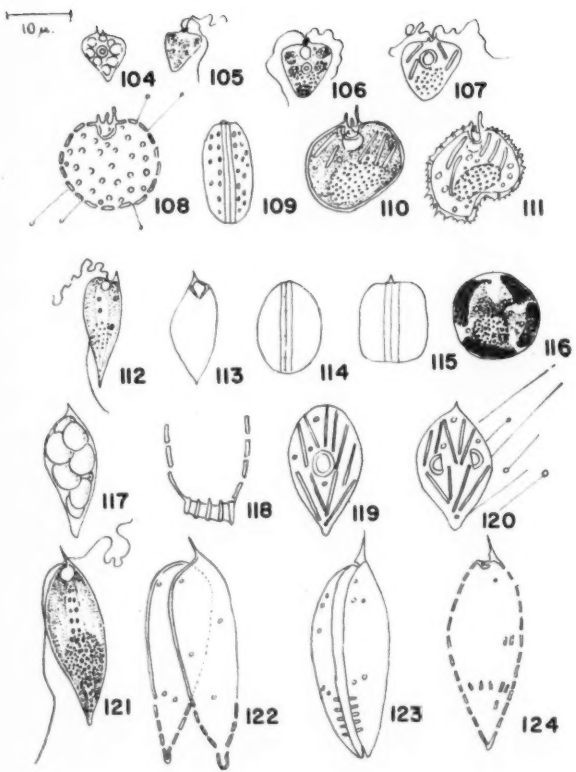
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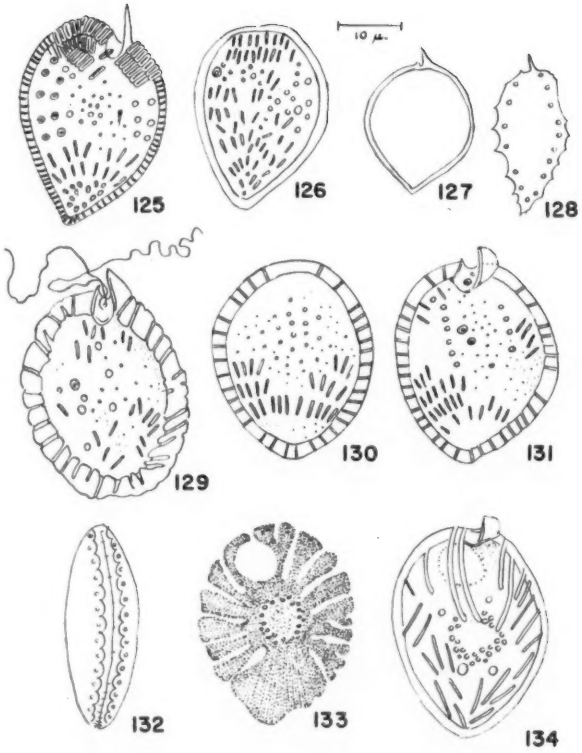
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THE ACTION OF DISODIUM VERSENATE ON THE EPIDERMAL CELLS OF LIVING BRASSICA ROOTS[1]

R. G. H. CORMACK

Abstract

This paper describes the action of the metal-chelating reagent disodium versenate on the epidermis of living *Brassica* seedlings. Striking abnormalities were produced, characterized by the repression of root-hair formation and by swelling and deformity of the epidermal cells. The results of the present study are briefly discussed in the light of evidence obtained by use of the electron microscope.

Introduction

Investigations dealing with the initiation and development of root hairs have led invariably towards a greater exploration of the epidermal cell walls. Most attempts to elucidate the chemical composition of the epidermal cell walls have involved microchemical and differential solubility tests for cell wall materials (3–8, 20, 24, 26). Another approach, introduced by the present writer, has been the use of various culture solutions designed either to stimulate or to hinder the change to calcium pectate in the elongating walls. It was introduced on the assumption that elongating root epidermal cells utilize calcium from their environment or that stored in the seed. The experiments to test the effects of calcium solutions, excessive acidity, excessive alkalinity, ammonium oxalate, and solutions of pectic enzymes were particularly convincing (3–11). The results reported here concern the effect of still another culture solution, disodium versenate, a very active chelating reagent for calcium and other metal ions involved in the development of the epidermis of *Brassica* seedling roots.

A special feature of *Brassica* seedling roots, and one that makes them interesting material for experimentation, is the differentiation of the epidermis into alternating rows of short and long cells. Normally the short cells form the hairs while the long cells remain hairless (3, 8). Differences in behavior between short and long cells have been attributed to differences in metabolism and food supply (3, 6, 7). Striking abnormalities were observed when *Brassica* seedlings were grown in solutions of ammonium oxalate and in solutions of pectic enzymes (3, 10, 11). The long cells were most affected, becoming greatly swollen, misshapen, and variously separated from each other. The short cells usually maintained their normal shape and arrangement and occasionally formed short hairs. The abnormalities were attributed to instability of the cementing pectic layer while the cells were still elongating. Removal of treated roots to a calcium solution or to tap water always resulted in marked recovery, characterized by cessation of abnormal development and by the immediate development of long hairs (3, 8, 10, 11).

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The present paper describes the growth of *Brassica* seedling roots in dilute solutions of disodium versenate[2] (Bersworth Chemical Co., Framingham, Mass., U.S.A.). It also compares the effectiveness of this reagent in producing abnormalities in the surface layer with that of ammonium oxalate and pectic enzyme solutions. Although Majumder and Dunn (19) have demonstrated the effect of various metal-chelating compounds on the growth of corn seedlings, the present writer knows of no detailed study of their effect on root structure. The experiments dealing with the effect of disodium versenate are still in progress. However, the results reported here are consistent and impressive enough to warrant publication at the present time.

Materials and Methods

The method and experimental conditions of the present study were essentially the same as those described by the writer in earlier papers (3, 11). Once again, seedlings of rape (*Brassica napus* var. *oleifera*) were chosen as the experimental material. Following germination on moist filter paper, the roots of a dozen seedlings some 2–3 mm in length were placed through small holes in pieces of stiff, paraffin-coated paper and floated in tap water in small beakers. Several hours later, when the roots showed a few millimeters of new growth in tap water, the floats were transferred to the experimental solution where the seedlings were allowed to grow for another 24–48 hours at room temperature. At the end of that time the roots were removed from the solution, measured, and examined under the microscope. As in earlier studies the usual procedure was to examine only half the roots and to transfer the float bearing the remainder to a beaker containing a saturated solution of calcium sulphate or tap water. A number of different versene compounds were tested, but to date disodium versenate has given the best results.

Observations and Conclusions

Up to the present time all attempts to grow *Brassica* seedling roots in dilute solutions of disodium versenate in distilled water have been unsuccessful. However, it was found that they would grow in dilute solutions of this chemical in tap water. The range of concentration which would allow for growth and yet produce striking epidermal abnormalities was exceedingly narrow, namely 0.015% to 0.020%. At the end of about 48 hours in the disodium versenate solution the roots measured 5 to 20 mm, about half the length of control roots in tap water. All treated roots had an unusual appearance and when examined under the microscope they showed two distinct regions: a short, upper normal region and below this a much longer deformed region. The upper normal part was developed in tap water prior to immersion in the disodium versenate solution. It was densely covered with root hairs, and with the exception of some plasmolysis did not appear to be adversely affected by the culture solution. On the other hand, the lower and much longer

[2]Disodium salt of ethylenediaminetetraacetic acid.

portion of the root, which had developed in the disodium versenate solution only, was obviously narrower and deformed. In general, it was characterized by the repression of root-hair formation and by marked deformity of the epidermal layer. As a rule the top of the deformed region was hairless, discolored, and severely cracked for a variable distance, indicating that on first contact with the versenate solution the meristematic cells were affected as well as the surface cells. Otherwise the treated roots resembled almost exactly roots grown in ammonium oxalate solutions (3). Wide variation occurred, but here again, as with roots grown in ammonium oxalate, it was possible to classify roots treated with disodium versenate into three types based on root-hair development: roots sparsely covered with a scattering of papillae and short hairs, roots that were hairless or practically hairless from the top to the tip, and roots with alternating hairless regions and regions of papillae and short hairs.

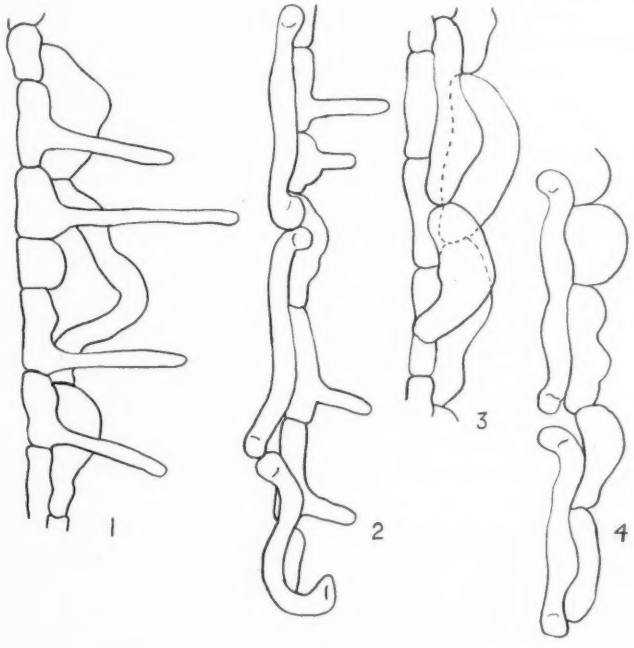
The degree of abnormality varied from root to root but the shape and arrangement of the epidermal cells were modified to some extent in every root. Freehand drawings made to scale, Figs. 1–4, are characteristic of the abnormalities consistently observed. As was true of roots grown in ammonium oxalate solutions, the long cells were most affected and assumed all manner of peculiar shapes. Swollen long cells were more numerous than separated long cells. Some separation of the long cells occurred but not nearly to the same extent as in roots grown in solutions of pectic enzymes (10, 11). Although it is difficult to generalize, it appeared that the long cells were most swollen and deformed in the same regions where the short cells formed papillae or very short hairs (Figs. 1, 2). Some of the short cells were swollen. The hairs measured 25–250 μ in length and were usually much wider at the base than those developed in tap water. Marked deformity also occurred in regions where the short cells were hairless (Figs. 3, 4). In some places only the long cells were swollen or separated while the short cells were more or less normal in shape and arrangement (Fig 3). In other places, the short cells were greatly swollen while the long cells were only slightly swollen but widely separated (Fig. 4). There were still other places where all the cells were so swollen and distorted that it was impossible to distinguish long cell rows from short cell rows.

As in earlier experiments in which ammonium oxalate solutions (3) and pectic enzyme solutions were used (10, 11), removal of the treated roots to tap water or to a saturated solution of calcium sulphate resulted in the resumption of normal growth. Almost immediately in the region of new growth the short cells produced long hairs and the long cells assumed their normal shape and arrangement in even rows. The region of new growth stood out in sharp contrast to the narrower deformed region above, which, as in earlier experiments, remained unchanged. Not all the roots responded to the changed conditions, indicating permanent injury to the apical meristem.

A feature of some interest not observed before in recovered roots was the unusual appearance of secondary roots developed from the deformed region

after the roots were transferred to tap water. The secondary roots were marked by a prominent hairless, pear-shaped swelling at the base, the surface of which was not differentiated into long and short cell rows. All the cells were slightly swollen with marked intercellular spaces at the corners. In most, the secondary root soon assumed its normal appearance with the regular arrangement of long and short cells and with the production of long hairs by the latter.

If calcium plays some role in wall formation, as the work of Roberts (24), Farr (14), Farr (15), McCoy (20), Burström (2), and many others suggests, then the marked abnormality that occurs when calcium is deficient or made unavailable might be expected (3, 8). The striking similarity of the effects induced by ammonium oxalate and by disodium versenate suggests that their modes of action are closely related. Both chemicals unite readily with calcium. The effects were similar in many respects to those induced by



FIGS. 1–4. A few rows of epidermal cells showing abnormalities produced in a 0.020% solution of disodium versenate. The apex of the root is toward the bottom of the page in each figure. ×125

FIGS. 1–2. Swollen and separated long cells, the short cells maintaining their normal arrangement in long rows. Most of the short cells form very short hairs.

FIG. 3. Greatly swollen and deformed long cells, the short cells are hairless but of normal shape and arrangement.

FIG. 4. Wide separation of the long cells and marked, swollen, and deformed hairless short cells.

pectic enzyme solutions except that in this case the action was on the pectic acid and not on the calcium. The abnormalities produced in the present study can be explained on the basis of the chelation of the calcium ions by the disodium versenate before they combine to form calcium pectate in the elongating cell walls. The solution was adequate to prevent all or almost all reaction between calcium and the pectic acid cementing layer of the long cells but not adequate to prevent calcification under the more favorable conditions presented by the short cells (3, 7, 8). As a result the short cells were least affected and quite often formed short hairs.

Roberts' (24) theory of the mechanism of root-hair development suggests that root hairs are evaginations produced by internal pressure on weaker portions of an unequally hardened cell wall. Evidence obtained by the present writer (3–11) shows that the hardening process is associated with the incorporation of calcium into the outer pectic wall layer of the epidermal cells and that the capacity to form hairs is dependent upon the rate at which calcification takes place. The same process of calcification that follows gradually along cell walls from the basal end of the cell to the apical end continues to occur uninterrupted behind the advancing root-hair tip once that structure begins to push out. In a recent paper on the growth mechanism of root hairs Ekdahl (13) concedes that calcium may promote the development of root hairs and may be incorporated into the hair-wall constituents, but questions whether a gradual calcification of an outer layer of pectic substances constitutes the main process determining the apical growth of root hairs. For this reason he favors the view that the hair wall is hardened by a formation of new cellulose strands, which are formed in the primary walls of the apices or laid down on the inner surface of the maturing hair wall. There is no disputing the presence of cellulose in the epidermal cell walls (3, 8, 20, 24), nor is there any question of its microfibrillar structure as revealed by the electron microscope (1, 17, 18, 21, 22, 23, 25, 26). However, it should be pointed out that, in relation to the development of root hairs in *Brassica* roots, cellulose occurs in the walls of the hairless long cells as well as in the walls of the hair-forming short cells. This observation strongly suggests that more is involved in the hardening process than the laying down of more cellulose strands.

The current view of primary wall structure, supported by the work of Frey-Wyssling (16, 17), Kerr (18), Preston (22, 23), and their schools of workers, is that it consists of a network or mesh of cellulosic microfibrils. The microfibrils are considered to be anchored, enmeshed, or embedded (as the case may be) in a mucilaginous matrix of non-cellulosic substances, mainly hemicelluloses and pectins. Two definite layers of microfibrils are recognized by Belford, Myers, and Preston (1) in the root-hair wall: an outer random layer of microfibrils termed the α-layer, and an inner oriented layer of microfibrils termed the β-layer. Unlike the clearly visible microfibrils of the β-layer, those of the α-layer are masked by large amounts of pectins and hemicelluloses. There is also some indication of chemical or physical differences between random and oriented microfibrils.

Growth of the primary wall is explained on the basis of the wall becoming softer as the result of an increase in the interfibrillar substances capable of swelling, or by the intercalation of new microfibrils (16, 17), or by changes in hydration of the pectic gel (18). Little has been said about maturation or hardening of the primary wall other than it involves the deposition of more cellulose strands (12, 13, 16, 18, 25). If changes in hydration of the pectic gel cause loosening of the cellulose microfibrils resulting in growth (18), it seems reasonable to assume that the gradual neutralizing of the pectic acid gel to calcium pectate would cause tightening of the cellulose microfibrils, bringing about maturity of the primary wall. A harder mucilaginous matrix would presumably assure firmer anchorage of randomized microfibrils embedded in it and provide a more solid bed for the deposition of oriented microfibrils. The marked swelling and deformity of the epidermal cells induced by ammonium oxalate and disodium versenate suggest insufficient calcium ions to effect a firm enough matrix for the anchorage of the cellulose microfibrils. The immediate cessation of abnormalities and development of long hairs when the treated roots are removed to a calcium solution strongly support this suggestion.

From this brief discussion of primary wall structure it would appear that the results obtained by electron microscopy support the early observation of Roberts (24) that the root-hair wall consists of an inner layer of cellulose and an outer layer of calcium pectate continuous with corresponding layers in the hair-forming cell. The presence of an outer layer of non-cellulosic mucilaginous substance or interfibrillar substance, no matter how thin, would explain the maceration of the epidermal cells by chemical reagents (3, 8, 20), and after treatment with pectic enzymes (10, 11, 13).

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STUDIES ON FERTILITY AND LATE BLIGHT RESISTANCE IN SOLANUM BULBOCASTANUM DUN. IN MEXICO[1]

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Abstract

Solanum bulbocastanum Dun. was collected extensively throughout its range in Mexico and Guatemala. Experimental self- and cross-pollinations showed that the species is highly self-sterile and consists of cross-sterile and cross-fertile individuals. Self-sterility may be due to the presence of incompatibility factors or to triploidy. Authentic hybrids were produced between *S. bulbocastanum* and *S. trifidum* Correll, and between *S. bulbocastanum* and *S. pinnatisectum* Dun.

After inoculation with race 1.2.3.4 of *Phytophthora infestans* three types of reaction were observed among 1148 seedlings of *S. bulbocastanum*: immunity with no perceptible lesions, resistance expressed by non-sporulating lesions of the hypersensitive type, and susceptibility indicated by large sporulating necrotic lesions. Seedlings resistant to an isolate of race 1.2.3.4 of Canadian origin did not always show the same level of resistance to a Mexican isolate of the same race. Varying degrees of field resistance were observed among seedling plants, while tuber-propagated plants were generally field immune. *Solanum bulbocastanum* is considered a mixture of resistant and susceptible genotypes.

Introduction

The Mexican wild potato species *Solanum bulbocastanum* Dun. has commanded the interest of potato breeders ever since it was found to be almost always immune to late blight of potatoes caused by *Phytophthora infestans* (Mont.) de Bary. Various workers (5, 10) have reported it immune after artificial inoculation with all their available physiological races of the pathogen. Also, Niederhauser and Mills (7) were unable to find authentic blight lesions on plants which had been exposed to an epiphytotic of the disease in the Valley of Toluca in Mexico during 1952.

Material of the species which has been available to potato breeders in the past has consisted of a few clones collected by Dr. Donovan S. Correll (3) in 1947 and by Dr. J. G. Hawkes (5) in 1949. Although with one exception these clones were immune to blight, attempts to produce hybrids between *S. bulbocastanum* and *S. tuberosum* were impeded by the fact that the immune clones were both self- and cross-sterile. The nature of the sterility was traced by Hunter (personal communication) to the fact that available clones in the U.S. and Canada were triploids with a chromosome number of 36. The

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single available diploid accession, P.I. 161156, was fertile but it segregated for susceptibility to blight according to Black and Gallegly (1). It therefore became a highly desirable objective to acquire by extensive collecting in Mexico a number of diploid, fertile, and blight-immune clones of the species.

Collections

S. bulbocastanum was collected throughout its range in central and southern Mexico and Guatemala during the rainy seasons of 1956 and 1957. In June and July it was possible to find plants in a vigorously growing condition suitable for transplanting, and living plants were brought back from the field in pots of soil. After they became established in the greenhouse they produced flowers in abundance and it was possible to perform many self- and cross-pollinations among the various clones. In late August and September the collections consisted of tubers and ripened fruits.

The species was found frequently around rock piles, in old lava beds, in ruins, and in the protection of cactus and maguey (*Agave* sp.) in semiarid locations at altitudes ranging from 5000 to 8400 ft above sea level. It was most interesting to note its relative profusion in and about certain archaeological sites. It was found on the pyramids of Cuicuilco and Cholula; in the ruins of the ancient city of Monte Albán, seat of the Zapotec culture in the Valley of Oaxaca; and at Zaculéu, Zacapulas, Utatlán, and Lake Atitlán in the highland Maya region of Guatemala. Naturally its presence here raises the question as to whether the species was ever cultivated. If we base our conclusions upon the results of cooking and tasting tests, it seems rather doubtful that the ancient Indian peoples used the tubers as food. It is more likely that *S. bulbocastanum*, in common with many other members of the genus *Solanum*, flourishes as a weed in the disturbed soil of the sites of the old civilizations.

Distribution of Fertile Clones

During 1956–57, 98 collections were made in the accessible areas of the range of the species. Preliminary attempts were made during 1956 to induce fruit and seed set by cross-pollinating clones from different locations in as many combinations as possible.

Three accessions, S-360, S-377, and S-398, from the Distrito Federal, and the states of Michoacan and Morelos, respectively, emerged as potential "tester" parents because of their high degree of cross-fertility. Subsequently they were used in detecting other fertile clones among the 1957 collections. All clones were self-pollinated as a matter of routine but fruit and seed set was encountered so rarely that the few berries which did appear were considered to be due to accidental cross-pollination. Approximately 70 collections were tested for fertility by using S-360 or S-377 or S-398 as male parents. Forty were found to be highly cross-sterile and 30 possessed an equally high degree of cross-fertility. The distribution of fertile clones shown on the map (Fig. 1)

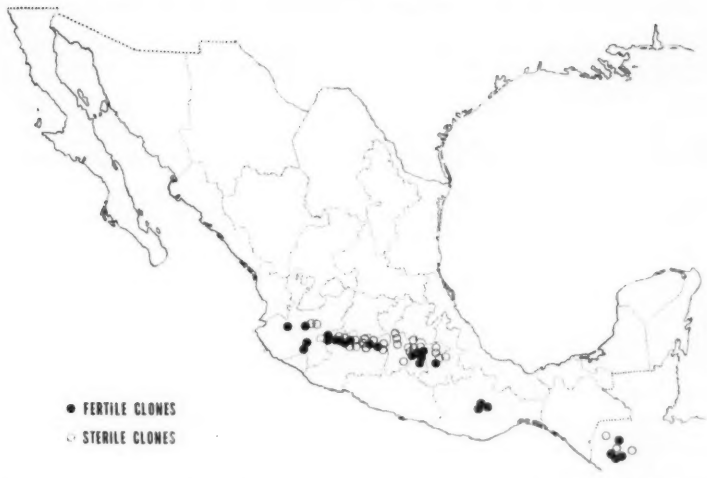

FIG. 1. Map of Mexico and western Guatemala showing distribution of fertile and sterile clones of *Solanum bulbocastanum* Dun.

is based upon these crosses. Table I shows representative data obtained from fruit and seed set on clones upon each of which it was possible to perform pollinations with all three of the tester clones as male parents.

TABLE I

Results of interclonal cross-pollinations with the clones S-360, S-377, and S-398 as male parents

	Male parents								
	S-360			S-377			S-398		
Female parent*	Fruit	Seeds	Seeds per fruit	Fruits	Seeds	Seeds per fruit	Fruits	Seeds	Seeds per fruit
S-359	3	238	79.3	2	96	43.0	1	73	73.0
S-362	2	134	67.0	3	207	69.0	4	227	56.8
S-376	2	5	2.5	5	2	0.4	3	8	2.6
S-395	3	147	49.0	7	353	51.7	4	240	60.0
S-396	5	156	31.2	2	110	55.0	3	109	56.3
S-397	3	241	80.3	5	340	68.0	2	76	38.0
S-420	4	254	63.5	1	25	25.0	3	89	28.7
S-463	4	140	35.0	6	100	16.7	6	220	36.7
S-479	1	2	2.0	0	0	0.0	2	5	2.5
S-480	0	0	0.0	0	0	0.0	6	3	0.5
S-481	0	0	0.0	0	0	0.0	1	3	3.0
S-482	0	0	0.0	0	0	0.0	5	7	1.4
S-483	12	195	16.3	0	0	0.0	9	250	27.8

*At least one flower cluster of from 8 to 12 buds was used for each cross-pollination of the female parent.

Observations of the Nature of Sterility in *S. bulbocastanum*

Fertile and sterile individuals have been found not more than a few kilometers apart and sometimes even in mixed populations (Fig. 1). It was not possible to perform chromosome counts on all the collections to determine if there was a cytogenetic cause for sterility. The chromosomes were counted in the pollen mother cells in young floral buds of 20 plants collected in the Pedregal de San Angel, an old lava bed located west of the University City in the Distrito Federal. The iron acetocarmine technique of Swaminathan and Howard (13) was used. The counts showed that we had found a large diploid population. It therefore appeared that the absence of fruits on plants in the field was not always caused by genetic sterility, but in most cases by unfavorable ecological factors such as excessive drought or poor soil conditions. Counts performed by Hunter (personal communication) had previously confirmed the presence of triploid plants at the northern end of the Distrito Federal only a few kilometers from the Pedregal de San Angel. Therefore, there is some evidence that *S. bulbocastanum* occurs at least in the Distrito Federal in both diploid and triploid forms.

The paucity of seed balls on plants found where ecological conditions seem to be favorable for growth and reproduction leads one to suspect the operation of a genetically controlled system of self- and cross-incompatibility factors. In dense populations the effects of such a system might be minimized by the activity of pollinating agents such as bees and other insects. However, the casual observation was made many times during collecting in Mexico that *S. bulbocastanum* seemed to have been decimated by the inroads of grazing animals and had been confined to the shelter of spiny xerophytes and the rough terrain of lava beds. With such a spatial separation of compatible genotypes, the effects of incompatibility factors may be quite significant in determining the quantity of seed set.

TABLE II

Fruit and seed set by crosses made between sister seedlings of *Solanum bulbocastanum*

Cross*	Fruits	Seeds	Av.
56-79 (3) × 56-79 (1)	2	73	36.5
56-64 (6) × 56-64 (1)	2	83	41.5
56-95 (9) × 56-95 (1)	3	150	50.0
56-107 (6) × 56-107 (1)	5	223	44.6
56-143 (3) × 56-143 (1)	5	285	57.0
56-110 (1) × 56-110 (3)	0	0	0.0
56-110 (3) × 56-110 (1)	0	0	0.0
S-362 (3) × S-362 (1)	0	0	0.0
S-362 (1) × S-362 (3)	0	0	0.0

*At least one flower cluster of from 8 to 12 buds was used for each cross-pollination of the female parent.

The presence of incompatibility factors in the diploid tuberous solanums has been reported previously by several investigators. The condition was found by Carson and Howard (2) in *Solanum lanciforme* Rydb., by Nath (9) in *S. Rybinii* Juz. & Buk, and by Pal and Nath (8) in *S. aracc-papa*, *S. caldasii*, *S. chacoense*, *S. jamesii*, and *S. subtilius*.

Evidence of intrafamily (self- and cross-incompatibility) in *S. bulbocastanum* came from a group of seedlings which had been selected for a study of the inheritance of resistance to known races of *P. infestans*. Data from crosses attempted between pairs of sister seedlings, representing seven families, are presented in Table II. Two of the seven families, 56-110 and S-362, apparently possessed factors for intrafamily incompatibility which completely prevented fruit and seed set in reciprocal crosses of sister clones. However, this intrafamily incompatibility was apparently overcome in the interfamily cross 56-110 (3) × S-362 (3), which was highly fertile. More intensive work is necessary to determine the frequency and the mode of inheritance of incompatibility factors in this species.

Late Blight Resistance in *S. bulbocastanum*

During the months of May and June, 1957, a large number of seedlings which had been obtained from the seed produced in the interclonal crossing program of the previous year were inoculated with two isolates of race 1.2.3.4. One of these was obtained originally from Mr. J. L. Howatt of the Plant Pathology Laboratory at Fredericton, New Brunswick, and the other was isolated during 1956 from a plant of *S. demissum* in the experimental plots in the Santa Elena Experiment Station, Toluca. Both isolates were single-spored and retested on Black's single-gene differentials prior to their use for the production of inoculum. At the time of inoculation the *S. bulbocastanum* seedlings were 6 weeks of age and possessed four or five true leaves. Inoculations were effected by dipping small pieces of absorbent cotton in zoospore suspensions of 10-day-old cultures of the pathogen, and then placing them on the leaves. Opposite leaves of each seedling were inoculated with race 1.2.3.4 of Canada and race 1.2.3.4 of Mexico respectively. The seedlings were then placed in a moist chamber for 36 hours at about 20° C, after which they were transferred to a greenhouse at about 20–25° C. After 4 days had elapsed from the time of inoculation, the dried pieces of cotton were removed from the leaves and readings of the degree and type of symptom expression were taken.

Three distinct types of reaction could be distinguished: first, immunity with no perceptible lesion of any kind; second, resistance expressed by pinpoint or dendritic lesions which produced no spores; third, susceptibility indicated by the presence of large necrotic areas which usually produced some spores after the leaves were placed in Petri dish moist chambers overnight. The reactions of 1148 seedlings are summarized in Table III.

Table III shows that *S. bulbocastanum* is not uniformly resistant to *P. infestans*. It was possible to segregate classes of seedlings immune, resistant,

TABLE III

Summary of reactions of 1148 seedlings of *S. bulbocastanum* to inoculation with Canadian and Mexican isolates of race 1.2.3.4 of *P. infestans*

Reaction to races 1,2.3.4		
Of Canada	Of Mexico	No. of seedlings
Immune	Immune	778
Immune	Resistant	135
Immune	Susceptible	21
Resistant	Immune	100
Resistant	Resistant	73
Resistant	Susceptible	28
Susceptible	Immune	5
Susceptible	Resistant	2
Susceptible	Susceptible	6

and susceptible respectively to race 1.2.3.4. Furthermore, seedlings carrying immunity to the isolate of race 1.2.3.4 from Canada did not necessarily carry immunity to race 1.2.3.4 of Mexico, and vice-versa. For example, 21 seedlings were immune to the Canadian isolate, but susceptible to the Mexican, and 5 seedlings were susceptible to the Canadian and immune to the Mexican.

The rainy season of 1957 afforded an opportunity to observe the resistance of the seedlings under field conditions in the Santa Elena Experiment Station in the Valley of Toluca, where late blight is usually present in epiphytotic proportions from July to September every year. The seedlings which had survived the inoculations with the isolates of race 1.2.3.4 were transplanted directly into 6-in. pots which were placed in rows in the experimental plots. About 25% did not survive the shock of transplanting and the method of planting whole tubers of wild species directly in pots in the field is therefore recommended. However, it was possible to make readings of blight incidence on 743 plants at the end of September. The seedlings were classified by the six arbitrary field resistance classes used by Mills and Niederhauser (6). In Table IV, the field and moist-chamber reactions of 743 seedlings are summarized.

Little correlation is evident in Table IV between the resistance of a given class of seedlings to race 1.2.3.4 of either Canada or Mexico and the degree of field resistance shown during 1957. For example, the 1.2.3.4-immune group of 493 seedlings broke down after a season's exposure into 104 individuals which were apparently field immune, 385 showing lesions of varying frequency and size, and 5 which were all but killed by blight. Furthermore, two of the four seedlings susceptible to both isolates of 1.2.3.4 survived the epiphytotic with readings of "2". The authenticity of the blight symptoms was established by making isolations from sample lesions. The identity of the races obtained is discussed in another publication (4).

Tubers of each of 27 clones of *S. bulbocastanum* collected during 1956 were also planted in pots in the Campo de Santa Elena. The plants from these

TABLE IV

Summary of the field behavior of 743 seedlings of *S. bulbocastanum* under conditions of an epiphytotic of late blight

(Santa Elena Experiment Station, Toluca, Mexico, 1957)

Reaction to races 1.2.3.4		No. of seedlings in arbitrary field resistance class*						
Of Canada	Of Mexico	0	1	2	3	4	5	Total
Immune	Immune	104	155	200	29	5	0	493
Immune	Resistant	23	22	44	10	0	0	99
Immune	Susceptible	2	3	4	1	0	0	10
Resistant	Immune	9	14	24	17	2	3	69
Resistant	Resistant	6	14	16	12	2	3	53
Resistant	Susceptible	2	2	6	0	1	0	11
Susceptible	Immune	0	0	1	1	0	0	2
Susceptible	Resistant	0	0	1	1	0	0	2
Susceptible	Susceptible	0	0	2	0	0	2	4

*0, none; 1, one to five lesions; 2, slight; 3, moderate; 4, severe; 5, dead.

tubers flourished throughout the season and with one exception, S-420 from the state of Oaxaca, showed a blight reading of "0" or "1". This result suggests that the species possesses a certain degree of field susceptibility in the seedling stage, but practical immunity in tuber-propagated plants. Furthermore, the possibility of segregation and selection for resistance in seedling populations in the field cannot be overlooked.

Interspecific Crosses involving *S. bulbocastanum*

The ultimate objective of the investigation was, of course, to obtain hybrids whereby genes for resistance might be transferred from *S. bulbocastanum* to commercial varieties of *S. tuberosum*. It is realized that the transfer of genes may be a very complicated process or even impossible by a direct cross between the two species. It may be necessary to use bridging species or hybrids whereby *S. bulbocastanum* genes can be "funnelled" through a series of interspecific hybrids to *S. tuberosum*. In the hope of finding useful "bridges" a number of crosses were attempted among *S. bulbocastanum* and other diploids. The preliminary results of this work are shown in Table V.

TABLE V

Fruit and seed set in interspecific crosses involving *S. bulbocastanum* and *S. tuberosum*

Female parent	Male parent	Fruits	Seeds
S. bulbocastanum	*S. trifidum*	3	135
S. trifidum	*S. bulbocastanum*	2	143
S. bulbocastanum	*S. pinnatisectum*	2	5
S. pinnatisectum	*S. bulbocastanum*	3	62
S. pinnatisectum (4×)	*S. tuberosum*	3	27

During 1957, seeds from the *S. bulbocastanum* × *S. trifidum* and *S. bulbocastanum* × *S. pinnatisectum* crosses were germinated and plants which seemed to be authentic hybrids were raised. The latter cross is not new, because Swaminathan (12) produced it with the aid of a technique of cutting off the stigmas and providing the germ tubes of the pollen grains of the male parent with nutrient in the form of a sucrose-agar medium. The crosses performed in Mexico needed no treatment. Unfortunately, the seeds produced in the *S. pinnatisectum* × *S. tuberosum* cross failed to germinate.

Some 700 seeds of *S. bulbocastanum* were treated with colchicine by the method described by Swaminathan (11), and from the surviving seedlings, about 30 plants were selected and grown to maturity. Some of these showed evidence of chromosome doubling, such as changes in the ratio of length to width of leaf, increase in size of floral parts, and self-fertility. Some of the plants were used in attempts at hybridization with tetraploid species, such as *S. stoloniferum*, *S. pinnatisectum* (4×), and *S. tuberosum*. So far, repeated efforts have not been successful.

Discussion

The occurrence of such a high percentage of sterile clones in the field in proximity to fertile clones seems unique in *S. bulbocastanum*. Although at the present time there is little evidence upon which to draw conclusions it seems reasonable to assume that the fertile clones are diploids and the completely sterile ones triploids. If this is true, then the question of the origin of so many triploids is raised. There is little phenotypic evidence of allotriploidy, because the tetraploid species within the range of *S. bulbocastanum* which might act as parents in the case of a putative allotriploid arising from a tetraploid × diploid cross are all compound-leaved species. The compound leaf character is apparently dominant to simple leaf in the successful hybrids *S. bulbocastanum* × *S. trifidum* and *S. bulbocastanum* × *S. pinnatisectum*. Autotriploidy, brought about by the frequent production and functioning of unreduced male and female gametes, is a possibility. This condition, conferring sterility, combined with the operation of cross-incompatibility factors of the kind demonstrated in Table II, may have seriously reduced the regenerative ability of the species under existing ecological conditions.

The series of inoculations of seedlings with Canadian and Mexican isolates of race 1.2.3.4 of *P. infestans* revealed two interesting facts: one, *S. bulbocastanum* is not uniformly immune or even resistant to late blight; two, race 1.2.3.4 of Mexico is not identical with race 1.2.3.4 of Canada. The identity of the races isolated from *S. bulbocastanum* by Graham, Niederhauser, and Romero (4) corroborates the latter finding. It seems apparent once more that race determinations are dependent upon the available range of differential hosts. If the host range can be extended, then additional races may be distinguished.

Genes conferring immunity to certain races of the late blight pathogen are present in *S. bulbocastanum*. The condition of a mixture of resistant and

susceptible genotypes has been noted by Black and Gallegly (1) in other species such as *S. verrucosum*, *S. cardiophyllum*, *S. pinnatisectum*, and *S. polyadenium*. The combination of resistance genes from *S. bulbocastanum* with resistance genes from *S. demissum* in *S. tuberosum* varieties would broaden the germ plasm basis for resistance, and perhaps would lessen the chance for the appearance of a race of *P. infestans* capable of attacking the genotypes developed in a breeding program utilizing this germ plasm. How the genes are to be introduced into *S. tuberosum* or how they would be affected by the backcrossing method is not known at the present time.

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THE FUNGICIDAL ACTIVITY OF METHYL BROMIDE[1]

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Abstract

Spores of *Alternaria solani* carried on glass fiber filter disks were employed in a study of the fungicidal activity of the fumigant, methyl bromide. Following exposure to the fumigant the disks, carrying the spores, were placed in a spore germination medium and the results subsequently evaluated by direct mortality counts. Linear probit-log dose regression lines were obtained by this procedure. The effectiveness of the fumigant was found to be dependent on the vapor pressure of water in the atmosphere and to be independent of temperature. The results suggest that humidity operates through its effect on spore hydration.

Introduction

The insecticidal, nématocidal, fungicidal, and bactericidal properties of methyl bromide are well known. As with other fumigants, two factors, namely, inherent biological activity and substrate interaction, determine its effectiveness in practice. Both are affected by the conditions prevailing during fumigation. Chisholm and Koblitsky (1) found that different soils sorbed different amounts of methyl bromide and further that sorption decreased as the moisture content of the soil increased. Similarly Monro (2) demonstrated a decrease in sorption by jute fibers and a corresponding increase in the rate of penetration into bales of jute bags as moisture content increased. This change was accompanied by a parallel increase in the susceptibility of his test insect, the cadelle. In contrast, Somade (5) showed that the sorption of methyl bromide by groundnut seeds increased with increasing moisture content and that germination injury increased accordingly. Fungi and bacteria were, however, controlled by fumigating dry seeds. Munnecke and Ferguson (3), studying nursery soil fumigation, concluded that 4 lb of methyl bromide per 100 cu. ft of soil was necessary to control the variety of soil-borne plant pathogens that they studied but reported that growers sometimes achieved good results with a much lower dosage. Such discordant findings are not surprising in view of the complexity of the problem.

Ludwig and Sampson (4) reported on a method of assaying antifungal activity under substrate-free conditions. This procedure involved the exposure of spores of *Alternaria solani* to the fumigant and the measurement of results by their subsequent ability to germinate. Standard straight line dosage response curves were obtained by this method with ethylene oxide and chloropicrin. The ability of the fumigants to kill the spores was profoundly affected by the humidity prevailing in the fumigation chamber.

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The results reported in this paper deal with certain refinements of technique and the application of the method to a study of some of the factors affecting the antifungal activity of methyl bromide.

Methods

Spores of *Alternaria solani*, washed from the surface of potato-dextrose agar cultures, were again used unless otherwise indicated. These were either used directly or collected on filter papers, dried, and stored over anhydrous calcium sulphate at 2° C until required. In the latter event they were washed from the papers for use. Spores on filter paper were stored for as long as 6 months without loss of viability.

Ludwig and Sampson (4) exposed spores on disks of cellulose filter paper. Direct germination counts could not be made and the spores had to be washed from the paper and suspended in water drops to germinate. Later the paper was supplanted by sintered glass disks and direct germination counts made after removal from the fumigant and suitable incubation under moist conditions. To facilitate observation the germ tubes were stained with hot methylene blue. Here the disks were costly and the staining procedure laborious. These difficulties were overcome in the present study by using disposable glass fiber filter disks in place of the sintered disks and developing a simplified staining procedure. In practice a number of the 2.1 cm glass fiber disks were placed, smooth side up, on a sintered glass funnel on a suction flask connected to the laboratory vacuum line. A fixed volume of a standard suspension of *Alternaria* spores, calculated to give an ultimate 15 to 30 spores per low power microscope field, was then pipetted onto each disk and the excess water drawn off with the vacuum. The disks were then transferred to a vacuum desiccator and further dried by holding under 0.5 cm pressure for a further 2 hours. The spores at this point appeared badly shrivelled when examined under the microscope. Thorough rapid drying was found to be extremely important since the spores of *Alternaria* germinate very rapidly. In preliminary experiments with methyl bromide the results indicated a failure to get complete kill. This proved to be due to pregermination rather than to a failure of the treatment.

After treatment, the disks were placed in petri dishes on filter papers saturated with 0.05% orange juice in distilled water. They wicked up the solution but were not flooded. Following germination, spores and germ tubes were stained with fast green in lactophenol to facilitate counting. The procedure followed in staining consisted of placing the disks in a sintered glass funnel on a suction flask, flooding with the stain solution and draining off the excess, and then washing with 25% lactic acid and again drawing off the excess. The disks were then mounted in lactophenol on microscope slides and covered with cover slips. The spores on disks prepared in this manner could be readily studied under the low power of the microscope with transmitted light and since the preparations were semipermanent they could be examined at leisure.

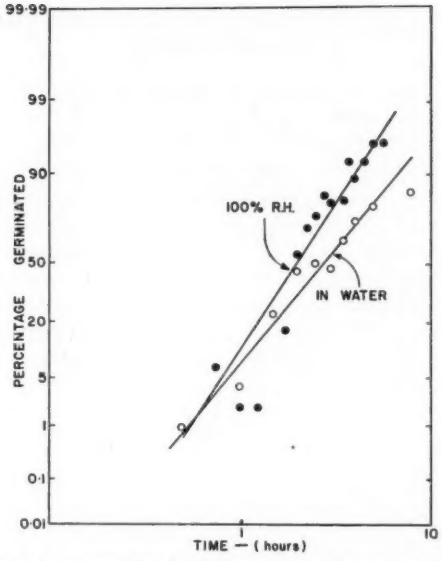

FIG. 1. Rates of spore germination in free water and a saturated atmosphere.

The rate of spore germination was determined under the conditions described above as well as in a saturated atmosphere without actual free water. This was done to determine the best "germination time" and to facilitate an evaluation of the effect of pregermination in connection with the ensuing experiments. The results obtained are shown in Fig. 1. In view of these results a standard germination time of 16 to 18 hours was adopted.

The disks were exposed to methyl bromide in 10-liter pyrex vacuum desiccators. The gas was introduced by first evacuating the vessels (5–10 mm Hg) and then admitting the gas from a cylinder in amounts calculated from manometer depression readings to give the desired concentrations as per cent by volume in air. Humidity ranges were established by returning the desiccators to atmospheric pressure with wet (100% R.H.) or dry (0% R.H.) air in proportions calculated to give the desired levels of humidity. The actual humidity prevailing was measured by means of a humidity sensing element contained in each desiccator.[4] The elements withstood repeated evacuation and pressure release without loss of sensitivity. Control was readily obtained at relative humidities below 90% by manipulation of the flow of wet or dry air, but it usually was necessary to introduce 1–2 ml of water into the desiccators to achieve 100% R.H. The accuracy of methyl bromide addition was checked with a thermal conducting gas analyzer.[5] The values obtained were relative and reasonably close to the calculated ones. Further, there was no drop in the gas concentration during the standard exposure period of 4 hours employed in this work.

[4]Eltronic laboratory hygrometer, Eltronics, Inc., Mayfield, Pa.
[5]Gas Master, manufactured by the Gow-Mac Instrument Co., Madison, N.J.

The Effect of Methyl Bromide on the Germinability of Spores of *Alternaria solani*

The effect of a series of dosages of methyl bromide on the germinability of spores of *Alternaria solani* was determined at 50% relative humidity and 25° C. The results are presented in Fig. 2. As expected a linear log-probit regression line was obtained. Since the relative humidity of the air during treatment affected ethylene oxide and chloropicrin fumigations (5) so markedly, its effect on the activity of methyl bromide was studied in three series of experiments employing a range of humidities and gas dosages. These were conducted at temperatures of 23° C, 10° C, and 5° C respectively. In these experiments all humidity levels were compared at a single dosage on one day and the gas dosages covered on consecutive days. Variability due to spore batches was minimized by collecting a uniform lot of spores for each series and storing them on filter paper until required.

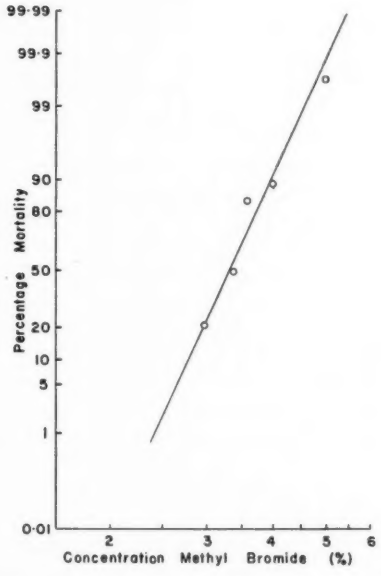

FIG. 2. Response of spores of *Alternaria solani* to methyl bromide at 50% relative humidity (mean of four experiments).

The ED_{50} values are plotted against water vapor pressure in Fig. 3 regardless of temperature. The products of water vapor pressures and effective concentrations are approximately equal except for the values obtained under low water vapor pressures where the ED_{50} could not be reliably determined. This indicates that activity is independent of temperature apart from its effect on the vapor pressure of the atmosphere.

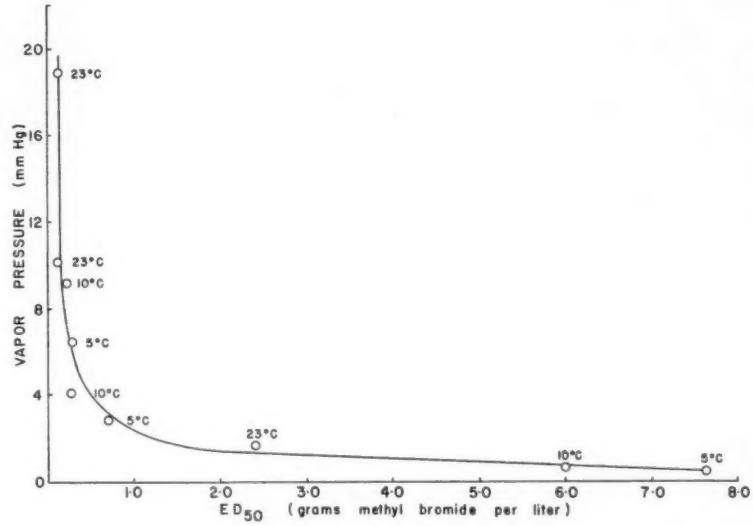

FIG. 3. The effect of atmospheric water vapor pressure on the activity of methyl bromide against spores of *Alternaria solani*.

TABLE I

The effect of methyl bromide on spores of *Alternaria solani* at various humidities and temperatures

Temperature	Relative humidity	Vapor pressure (mm Hg)	ED_{50} (g/liter)	Slope*
5	0–8	0 –0.5	190.0†	–
	45	2.9	18.0	7.5
	100	6.5	7.3	1.5
10	0–8	0 –0.7	150.0†	75.0
	45	4.1	7.0	3.0
	100	9.2	5.8	2.5
23	0–8	0 –1.7	60.0†	14.0
	49	10.2	3.0	1.1
	90	18.9	3.5	1.1

*Slope, $((ED_{50}/ED_{16})+(ED_{84}/ED_{50}))/2$.
†Values here were obtained by extrapolation and could not be achieved under atmospheric pressure.

An examination of Table I shows that the dosage response curves obtained become increasingly steep as the water vapor pressure is increased. Those for low and high vapor pressure actually crossed at low dosages. The effect of this on percentage kill is illustrated in Fig. 4. It explains earlier results where a fall-off in activity was noted at high humidities.

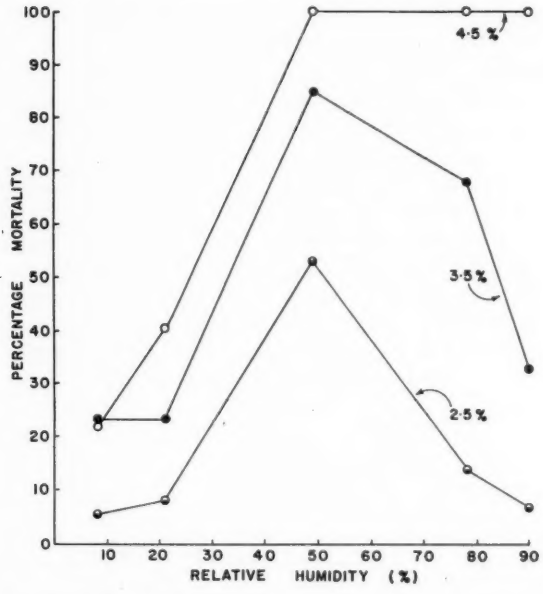

FIG. 4. The effect of relative humidity on the mortality of *Alternaria solani* spores induced by low doses of methyl bromide.

Spore Hydration and Germination at Various Vapor Pressures

The effect of humidity on the fungicidal activity of methyl bromide suggested that the degree of hydration of the spore might affect the fungicidal activity of this fumigant. A study was therefore made of the degree of spore hydration under various conditions of atmospheric humidity. In this study the degree of hydration was determined by equilibrating spores in microbeakers over either saturated salt solutions or standard sulphuric acid solutions in closed jars and determining their change in weight by weighing on a microbalance. The jars were rocked gently on a mechanical shaker during the equilibration period to prevent stratification. The results were recorded as a percentage increase over spores dried under vacuum over P_2O_5.

Parallel to these experiments spores of *Alternaria* carried on glass fiber disks were maintained in comparable atmospheres and samples were removed at intervals to determine degree of germination and to observe the appearance of the spores. In the latter case the disks were flooded with mineral oil immediately on removal to prevent further change in hydration.

The germination and hydration curves obtained are presented in Fig. 5. It will be noted here that the hydration curve is of the same general shape as the activity curve shown in Fig. 3. Results recorded for 100% and 99% relative humidities were not reliable and have been omitted since it was difficult

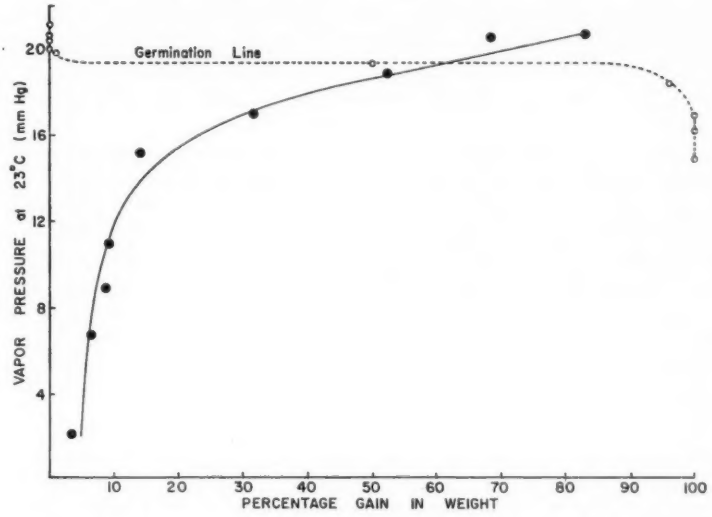

FIG. 5. The hydration and germination of *Alternaria* spores at various humidity levels.

to produce these conditions with any certainty and to be sure that free water had not condensed on the spores. The lower limit for germination was found to be around 87% relative humidity at 23° C.

Observations on the mineral oil mounts showed that the spores did not change from their original shrivelled condition unless exposed to free water even though they germinated.

Discussion

The technique originally developed by Sampson and Ludwig (4) for the examination of the antifungal activity of fumigants under substrate-free conditions was simplified by the adoption of glass fiber filter disks as spore carriers and the development of an improved staining procedure to facilitate direct germination counts. Spores of *Alternaria solani* were again used as the test agent since they withstand extreme desiccation without loss of viability and germinate rapidly and completely on suitable rehydration. Spore germination was found to occur in the absence of free water and in fact some germination occurred at room temperature in atmospheres with a relative humidity as low as 87%. This may be of importance in relation to the epiphytology of the early blight disease and merits further study in this regard. Since spore germination is very rapid, extreme care must be taken, in work such as that reported here, to avoid artifacts created by germination prior to treatment.

The slopes of the dosage response curves for methyl bromide increased with increasing relative humidity and actually intersected in the lower kill range.

This explains the apparently anomalous finding that low dosages appear to be more effective at intermediate relative humidities than they are at saturation. With a very susceptible organism it is conceivable that control might be obtained by a low gas dosage under dry conditions but not under wet conditions.

The effect of relative humidity on activity appears to be directly related to spore hydration and to be independent of temperature except in so far as the latter affects the water vapor pressure in the atmosphere. This seems to be analogous to the behavior reported by Somade (5) for groundnut seeds where phytotoxicity increased with increasing moisture content.

The behavior of chloropicrin reported on earlier by Sampson and Ludwig (4) appears to be similar to that described here for methyl bromide. This is not surprising since both gases are relatively water insoluble. Their behavior in relation to moisture differs from that of the water-soluble gas, ethylene oxide.

Acknowledgments

The authors are indebted to Dr. H. A. U. Monro for continued interest and advice. The help of Mr. C. Unwin and Mr. J. Gates in the preparation of the figures is also gratefully acknowledged.

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NUCLEAR PHENOMENA IN THE LIFE CYCLE OF GANODERMA LUCIDUM (LEYSS. EX FR.) KARST.[1]

ANJALI SARKAR[2]

Abstract

Nuclear behavior in the life cycle of *Ganoderma lucidum* (Leyss. ex Fr.) Karst., a common polypore in West Bengal, India, has been studied. Each young basidium is binucleate and the nuclei soon fuse to form a syncaryon which undergoes three successive divisions, of which the first is reductional, to produce eight daughter nuclei. Four nuclei pass into the four spores, one into each, and the other four, remaining within the basidium, finally degenerate. Thus at first each basidiospore contains one nucleus which may or may not divide further. On germination, the spores give rise to primary mycelia with uninucleate or multinucleate cells. Secondary mycelia, formed by the union of two compatible primary mycelia, have nodose-septate hyphae in which most of the cells are dikaryotic although cells with more than two nuclei are not uncommon.

The chromosome number, counted from metaphasic plates, has been found to be $10 = 2n$.

Introduction

Our present knowledge of the cytology of the higher fungi is due to the outstanding researches of a number of investigators, such as Maire (10), Levine (9), Kniep (7, 8), Juel (6), Sass (13, 14, 15, 16), Wakayama (17, 18), and others. Their labors stimulated great interest among subsequent workers who have paid increasing attention to studies on the nuclear condition in the life cycles of fungi. Their efforts have resulted in good contributions to the knowledge in this field, particularly in the Hymenomycetes. Recently Olive (11) has presented an excellent review on the subject.

In India, very few species of Hymenomycetes have been studied cytologically and of these, not all have been studied in detail. Bose (4), the first to start research in the group, described nuclear conditions in the basidia of 11 species of polypores. Recently Banerjee and Mukherjee (1, 2) described nuclear details in two other species, *Marasmius campanella* Holterm. and *Stereum fuscum* (Schrad.) Quél. As far as the author is aware, these are the only records of cytological studies of the Hymenomycetes that have been done in India.

With a view to adding cytological knowledge for another member of the group, the present investigation of *Ganoderma lucidum* (Leyss. ex Fr.) Karst., a very common polypore in West Bengal, India, was undertaken. Bose (3) reported some cytological studies on *G. lucidum* but his work was concerned mainly with the secondary spores.

The present paper presents a more detailed study on the karyological phenomena of the fungus, including the nuclear features in the basidia, basidiospores, germinating basidiospores, and primary and secondary mycelia. The structural details of the fusion nucleus in its resting stage and in the following meiotic stages are also described.

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Materials and Methods

For the study of the basidia, hymenial regions of fresh fruit bodies of *Ganoderma lucidum* on living hosts in nature were used. Small portions of mature hymenial surfaces were fixed in the field in different killing and fixing reagents, including Bouin-Allen, Flemming (weak), 2BD (La Cour), and acetic–formalin–alcohol. Of these, the first, Bouin-Allen, yielded the most successful preparations. The materials were fixed in two lots and in different intervals of time, one between 12:30 and 3:00 P.M. and the other between 12:30 and 2:00 A.M. Washing, dehydration, and imbedding were done by following the standard schedule. Sections 12 to 14 μ in thickness were found to be suitable for observation. Of the stains, Heidenhein's iron alum haematoxylin and crystal violet were tried and the former was found to yield most satisfactory results.

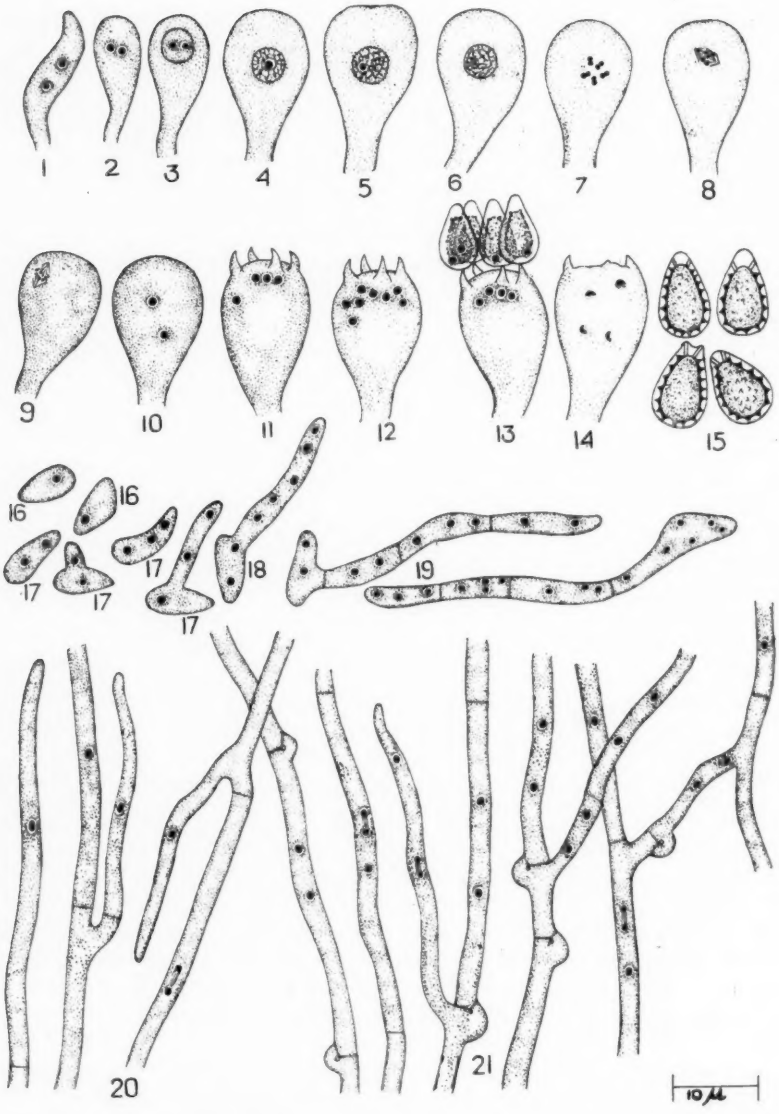
The study of the nuclear conditions in spores, germinating spores, and primary and secondary mycelia was made from preparations on slides, following Kniep's agar film technique with certain modifications. The composition for the medium was as follows: malt extract 2.5%, agar 2%, and distilled water 1000 cc. The constituents were dissolved in the distilled water, filtered through a filter paper in a steamer, tubed, and sterilized in Koch's sterilizer at 100° C in the usual way. When required, the medium was liquefied by heating the tubes in a water bath, and a few drops of the medium were placed on a warm sterile slide and spread to form a thin film, with care being taken that the film did not reach the edges of the slide. As soon as the agar film on the slide solidified, it was inoculated with the mycelium, either primary or secondary. For the study of the nuclear condition in spores and germinating spores, spores were allowed to fall from fresh sporophores directly on agar films and germinate there under sterile conditions. When the spores, germinating spores, or mycelia reached the desired stage, the agar films were dried partially at the margins only in dry, covered Petri dishes. Special care was necessary in drying the agar medium, because if it was dried too much, the mycelium would have been damaged, and if it was not dried sufficiently, there was the possibility of its being washed off during the washing process. The principal fixing fluids used were Bouin-Allen, chromo-acetic fixative (1% chromic acid 25 cc, 1% acetic acid 10 cc, distilled water 55 cc), Sass' fixative, and acetic–formalin–alcohol, of which the first two gave the most satisfactory results. For staining, Heidenhein's iron alum haematoxylin solution was used, following the method suggested by Gwynne-Vaughan and Barnes (5).

Observations

Basidia

Mature basidia of *Ganoderma lucidum* are broadly clavate in shape, tetrasterigmatic, always bearing four spores. Young basidia, on the other hand, show all stages of transition ranging from slightly inflated, terminal hyphal cells to broadly clavate, mature ones. At the earliest stage, the young basidia are always binucleate with the two paired nuclei lying one above the other and

at a little distance apart from each other (Fig. 1) or lying side by side and very close together (Fig. 2). These nuclei resemble the nuclei of the vegetative cells in structure and are of the "homogeneous unexpanded" type, as defined by Pinto-Lopes (12). According to Pinto-Lopes, a nucleus of this type consists of a central, deeply stained chromatin body, separated from the nuclear membrane by a hyaline area. This conjugate pair of nuclei within the young basidium soon fuse to form a syncaryon. At the time of karyogamy, the young basidium becomes somewhat inflated, narrowly clavate in shape, and quite distinct in appearance from the usual hyphal tips. After the syncaryon is formed, it moves, in some cases, to the upper part of the basidium (Fig. 3). In the early stages of fusion the two nucleoli of the original conjugate pair can often be easily identified, lying within the common nuclear membrane. These two nucleoli ultimately fuse to form a large, deeply stained nucleolus (Fig. 4) situated either at the center or at one side of the nucleus. The fusion nucleus is very prominent and much larger in size than the vegetative nucleus. At the resting stage it consists of a deeply stained nucleolus, several deeply stained bodies on a feebly stained reticulumlike chromatin structure, karyolymph, and a nuclear membrane. Karyogamy is soon followed by the meiotic prophase. In the late prophase, these chromatin bodies become condensed and well marked, and finally threadlike chromosomes are formed (Figs. 5, 6). At this stage, the chromosomes stain only weakly and their number could not be ascertained with certainty. At the metaphase, the chromosomes appear much condensed and arrange themselves in five pairs at the equatorial region of the spindle (Fig. 7). From several metaphasic plates, the chromosome number of *Ganoderma lucidum* was found to be 10 ($=2n$). At this stage, the nucleoli and nuclear membrane are totally lacking and in no case were centrosomes in evidence. The orientation of the spindle within the basidium is not at all definite, being either transverse or obliquely longitudinal in the upper part of the basidium (Figs. 8, 9). From this first meiotic division of the fusion nucleus two daughter nuclei are formed (Fig. 10) which, after two successive, equational divisions, eventually produce eight daughter nuclei of the same size and structure (Figs. 11, 12). These are larger and more conspicuous than the ordinary vegetative nuclei and are of the "homogeneous unexpanded" type. The nuclei in the octonucleate stage of the basidium are variable in position but they mainly remain toward the upper part of the basidium, around a large central vacuole. Four sterigmata begin to develop at the apex of the basidium while it is in the quadrinucleate stage. When fully developed, the tips of the sterigmata gradually become swollen and enlarged and finally a spore is formed at the apex of each and nuclear migration from the basidium to the spore takes place. Four of the eight nuclei of the basidium move upwards toward the sterigmata and finally migrate through them, one through each sterigma, into the spores (Fig. 13). Thus a spore of *Ganoderma lucidum* is always initially uninucleate, the nucleus being located at the broad and rounded base of the spore. In a mature spore, this nucleus divided only once, to form two daughter nuclei. After the formation and discharge of the spores from the sterigmata,



FIGS. 1 to 21. Nuclear phenomena in basidia, secondary spores, germinating spores, and primary and secondary mycelia of *Ganoderma lucidum* (For explanation *vide* text).

the four nuclei that still remain within the basidium soon degenerate and finally the old basidium with the degenerated nuclei collapses (Fig. 14). The position of the nuclei in mature basidiospores could not be observed on account of the verrucose nature of the spore walls (Fig. 15).

Germinating Spores

The writer experienced great difficulty in germinating basidiospores of *Ganoderma lucidum* on agar media; the percentage of germination was found to be 1 to 2% or less. Even when germination occurred, the germinated basidiospores were so few and so far apart in the preparation on an agar film that it was impossible to get all the stages of germination within the field of the microscope for cytological observation. To obviate this difficulty, the nuclear phenomena in the early stages of spore germination were studied from secondary spores which, when fully mature, resembled basidiospores exactly in structural detail.

The secondary spores of *G. lucidum* were obtained from fructifications formed in culture. These spores were unicellular, thin-walled, more or less hyaline, and at first without any evident endospore. In this condition they germinated readily on 2% malt agar medium and the percentage of germination was very high.

Most of the secondary spores were uninucleate with the nucleus situated, in most cases, at one side of the broad end of the spore (Fig. 16). Occasionally two or three nuclei were observed within the spore (Fig. 17). This binucleate or trinucleate condition of the spore could be interpreted as due to divisions of the original spore nucleus. The trinulceate condition was rather rare and the spore, in that condition, became somewhat elongated. The germination of a spore started with the formation of a short germ tube from any point on the spore wall. Only one germ tube was produced by a spore at one time; in no case were two germ tubes found to originate from it. As soon as the germ tube was formed, one or two nuclei immediately migrated into it. With the elongation of the germ tube, these nuclei divided repeatedly without accompanying wall formation so that the germ tube became coenocytic with several nuclei scattered within it (Fig. 18). When the germ tube attained a length of 20 to 30 μ, cross septa began to appear somewhat irregularly, forming multinucleate hyphal cells (Fig. 19). In some cases, the first septum was formed at the junction of the germ tube and the spore but this was not always the case. In the early stages of germination, regular monokaryotic cells were not formed but in the primary mycelia developed from single spores, each hyphal cell was usually in the uninucleate condition, although cells with more than one nucleus were encountered (Fig. 20). This binucleate or multinucleate condition may be explained as due either to irregular wall formation or to division of the original nucleus in each cell.

Similar observations were made on the nuclear condition in secondary mycelia obtained by mating primary monosporous mycelia of opposite sexes. The cells of the secondary mycelia were 'dikaryotic' in the sense that most of the cells contained two nuclei, although cells with two to four nuclei were not

uncommon (Fig. 21). Clamp connections were not formed regularly at each septum and, as a result, hyphae bearing simple septa as well as clamp connections were quite frequent.

The nuclei in the germinating spores and in the primary and secondary mycelia all were of the "homogeneous type" as stated before, and in most cases the nuclei were comparatively small with respect to the lumen of the hyphal cell. Stages in the division of vegetative nuclei were very rare in all preparations.

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FINE STRUCTURE IN CELLS OF PEA AND WHEAT EMBRYOS[1]

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Abstract

To provide a basis for relating biochemical findings on isolated cell fractions to cytological structure *in situ*, embryos of pea and wheat were fixed with osmic acid, sectioned, and observed in phase-contrast and electron microscopes. The nuclei of all cells were similar, showing nuclear membranes, chromosomes, and prominent nucleoli. The cytoplasm contained highly developed structure which presumably reflected the incipient growth condition of the cells. Several cytoplasmic components were common to both embryos: small dense granules, endoplasmic reticulum, mitochondria, presumed proplastids, amyloplasts, irregular bodies, plasma membranes, and plasmodesmata. The small dense granules, presumably ribonucleoprotein particles, occurred profusely, both free and in association with extensively developed endoplasmic reticulum. These particles are probably responsible for the microsomal fractions obtainable from embryos and seedlings. The mitochondria were usually relatively small (0.25–0.5 μ diameter) although groups of very long (5 μ) ones were occasionally found. Bodies resembling mitochondria in size and shape, but lacking cristae, were present and represent either immature mitochondria or proplastids. Reserve material occurred as starch in structurally complex amyloplasts and possibly as protein in the irregular bodies. In addition to these structures cells of the wheat embryos remote from the meristems contained prominent cytoplasmic bodies classified as "dense" and "thick-walled". The dense bodies probably represent stored lipids while the significance of the thick-walled bodies, which showed a variety of forms, is unknown.

Introduction

The protoplast of plant cells has been thoroughly studied with the optical microscope and an extensive array of cellular components has been described (4, 15). Unfortunately, two objections apply to much of this work. First, since relatively drastic preparative procedures were used and the structures studied approached the limits of resolution of the light microscope, the reality of many of the components described remains open to question. Second, except for obvious elements such as chloroplasts and chromosomes, little attempt was made to relate chemical composition or physiological function to observed structures. Recently, electron microscope studies have begun to provide more exact descriptions of structure within plant cells (5, 6, 7, 11, 12, 14, 20) and attempts have been made to relate this structure to biochemical composition and function (7, 12).

A series of studies has been undertaken in an attempt to correlate subcellular structures of plant embryos with their biochemical behavior. As a part of these studies this paper presents a survey of fine structure within cells of pea and wheat embryos. The results provide a basis for relating biochemical findings on isolated cellular components (22) to cytological structure *in situ*.

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[2]National Research Council.
[3]Canada Department of Agriculture.

Materials and Methods

Embryos of pea (*Pisum sativum*, Arthur variety) and wheat (*Triticum vulgare*, Chinook variety) were isolated by the procedure of Johnston and Stern (9). The embryos were placed on damp filter paper for 4 hours and then fixed for 2 hours at 0° C in buffered (pH 7.4) 2% osmium tetroxide (17), 0.6 *M* with respect to sucrose. Embryos were fixed intact for phase-contrast microscopy and cut into small pieces (ca. 0.5 mm^3) in the fixative for electron microscopy. After fixation the tissue was kept at 0° C and washed twice with 0.6 *M* sucrose and placed in 70% ethyl alcohol. The tissue was then warmed to room temperature, dehydrated, embedded in methacrylate (3 parts butyl to 1 part methyl), and sectioned. Sections were cut 2 μ thick for phase-contrast microscopy and with gray to silver interference colors for electron microscopy.

Observations

Phase-Contrast Microscopy

In the optical microscope, cells of the pea embryos were fairly homogeneous with respect to cytological structure. Cells near the apical meristems were small, regularly shaped, and showed only a nucleus and finely particulate cytoplasm. Toward the base of the epicotyl and hypocotyl the cells were slightly larger, less regular in shape, and contained granular cytoplasm with prominent starch (I_2–KI positive) bodies (Fig. 1). The starch bodies progressively increased in size in cells toward the base of the cotyledons (Fig. 2).

Cells in the meristematic regions of the wheat embryos were similar to those in the same regions of the pea. However, cells near the base of the epicotyl and hypocotyl and in the coleoptile, coleorhiza, and scutellum showed a diversity of prominent particles in the cytoplasm (Figs. 3 and 4). These particles were usually spherical, varied greatly in size, and were not I_2–KI positive. Only occasional small starch grains were detected in the wheat cells by I_2–KI staining.

Electron Microscopy

Figure 5 is a low magnification picture of a section of a cell from near a meristematic region of a pea embryo. All pea cells observed had this general appearance except that the amyloplasts (A) were larger in cells remote from the meristems. In the wheat embryo, cells near the meristems had a similar appearance except that the amyloplasts were generally less evident. On the other hand, in agreement with the phase-contrast results, wheat-embryo cells remote from the meristems had very distinct structural differences as shown in the low magnification views of Figs. 6 and 7. These differences will be discussed below.

The nuclei (N, Figs. 5, 6, 8) of all types of cells in both embryos were similar to each other and to those described in other tissues, e.g. (5). A nuclear membrane (Nm), which only rarely appeared as double, surrounded a relatively transparent nucleus. The general lack of double nuclear membranes may be real but is more probably a result of inadequate fixation. The

nucleus contained irregularly shaped dense bodies (C), presumably sections of chromosomes, and a prominent nucleolus (Nu). The nucleolus appeared as a circular body, without a delimiting membrane, composed of moderately dense heterogeneous material which lacked obvious ordered arrangement. The nucleoli of pea cells were generally larger than those of wheat and often contained clear central areas (Cl) similar to those reported for nucleoli of root-tip cells (10). The small dense structures found in root-tip nucleoli (10) were not encountered in the embryo cells.

The cytoplasm of the cells showed a variety of structures. Components common to both pea and wheat cells may be roughly classified as follows:

1. Small dense granules.
2. Endoplasmic reticulum.
3. Mitochondria.
4. Presumed proplastids.
5. Amyloplasts.
6. Irregular bodies.
7. Plasma membrane and plasmodesmata.

The small dense granules, 10–20 mμ in diameter (G region, Figs. 8, 9, 12, 13), were similar to those described by Lund *et al.* (12) in corn roots. They were scattered in the cytoplasm although the frequency varied in different regions. These granules probably represent ribonucleoprotein particles (19, 23).

Various forms of endoplasmic reticulum (E, Figs. 5–9, 11, 16, 18) similar to those described in other tissues were encountered. Both individual lamellae and bundles of lamellae were present, the latter being more common in wheat cells. Small vesicles of various shapes, presumably representing sections across the reticulum, were commonly seen (V, Fig. 9). Some of the membranes were smooth-surfaced while many had associated small particles resembling the ribonucleoprotein particles described in both plants and animals (6, 13, 19). Small multilamellar structures similar to those found in other meristematic plant cells (11, 12) and to the dictyosome of animals cells (2) were seen occasionally (Fig. 10).

Mitochondria (M, Figs. 5–9, 11–13, 18–19) with the same general structure as those of other tissues, i.e., double outer membranes and internal cristae mitochondriales (5, 7, 12, 24), were found in cells of both types of embryo. Usually the mitochondria showed circular or oval profiles with diameters of 0.25–0.5 μ (Fig. 13), while in a few cells groups of very long (ca. 5 μ) mitochrondria similar to the chondriochonts described by Lance (11) were found (Fig. 11). Although the mitochondria were widespread within the cells they often occurred in localized groups (e.g., Fig. 17) while large areas of the cells contained none (e. g., region of the irregular bodies, I, Fig. 5).

In cells of both embryos, bodies similar in size and shape to mitochondria, but lacking internal cristae, were found (Figs. 12, 16). At least some of these bodies probably represent proplastids (11, 14) although it is possible that others are immature mitochondria (12).

Cells of both embryos contained amyloplasts (A, Figs. 5–7, 14, 15) reasonably similar in structure to those found in other plants (6, 14). The amyloplasts (Figs. 14, 15) consisted of an outer membrane, often appearing double, surrounding a diffuse matrix (Ma). Large bodies presumed to be starch grains because of their transparency (typical of carbohydrate material, e.g. cell walls in Figs. 5–7) lay in the matrix. They were bounded by a fairly dense membrane and often showed localized internal bands of less transparent material. Small dense granules (Dg), 5–8 mμ in diameter, occurred in the matrix. These granules were considerably more profuse in the amyloplasts of pea than of wheat.

Structures classified as "irregular bodies" (I, Figs. 5, 8, 13) were found in cells of both embryos. The great variety of shapes suggests that these bodies may represent sections of a complicated network. The bodies were delimited by a membrane and the interior contained material of moderate density. In pea the internal material was somewhat irregular in structure while in wheat it was homogeneous. In wheat (Fig. 13) the bodies were often found to narrow to dimensions approaching those of the endoplasmic reticulum. The irregular bodies were generally restricted to definite regions within individual cells (Fig. 5) and were not found in all cells.

The cytoplasm of all cells was bounded by a dense membrane (Pm, Fig. 12), about 10 mμ thick, appressed to the cell wall. Occasionally this membrane appeared double. Fine plasmodesmata (Pd, Figs. 6, 7, 12) penetrated the walls, usually appearing as double lines and infrequently, presumably when cut obliquely, as tubes. The large dense plasmodesmata described by Huber *et al.* (8) were also present.

In addition to the above structures which were more or less similar in both types of embryo, cells of the wheat embryos showed a number of unique cytoplasmic structures. These are loosely classified as "dense bodies" and "thick-walled bodies".

The dense bodies (D, Figs. 6, 7, 9, 12, 13, 17–20) were distributed throughout the cytoplasm but often occurred in higher frequency near the cell walls (Fig. 7). They were irregular in shape with diameters of 0.2–0.4 μ, and often appeared clumped.

The bodies classified as thick-walled (T, Figs. 6, 7, 17–20) showed a variety of forms. Although they are considered here as a group it is not certain that they are all members of a single structural species. The most common form consisted of a moderately dense membrane, usually circular in shape and approximately uniformly thick (75–150 mμ), surrounding a central region which was commonly transparent (Figs. 6, 17). Frequently circular bodies of the same density as the outer membrane occurred in the central cavity, either free (Fig. 7, top) or in contact with the membrane (Fig. 18). In another arrangement the outer membranes varied fairly regularly from thick to thin and were often broken and disturbed (Figs. 7, 19). Finally, large solid bodies of about the same density as the thick membranes of the other bodies

were often found (Fig. 20). The size of all of these bodies varied greatly: from less than one micron to several microns in diameter. Only one type of body was found in any one cell (Figs. 6, 7) and adjacent cells usually contained the same type of body.

Discussion

Mature plant embryos of the type studied here are complex structures containing both meristems and regions of partial cellular differentiation. Physiologically, however, all of the cells are distinctive because of their incipient condition; they have yet to undergo extensive growth following prolonged dormancy. It is interesting therefore to note the great number of subcellular structures present and to compare them with those found in other tissues. Many of the components of the embryo cells were basically similar to those in cells of growing or mature tissues, while others appear uniquely associated with the embryonic condition. Studies on growing roots (7, 12) would suggest that some of the structures become modified or disappear as growth proceeds.

The endoplasmic reticulum and presumed ribonucleoprotein particles appear to be among the most extensively developed structures of the cells. They are very similar to those found in actively metabolizing animal cells (19). It may be supposed that a major activity of a newly germinating embryo is the synthesis of proteins made possible by the presence of highly developed nucleoprotein structures. Probably the microsomal fractions isolated from pea seedlings which are capable of amino acid incorporation in vitro (25) are derived from the well-developed endoplasmic reticulum and ribonucleoprotein particles. The profuse ribonucleoprotein particles free in the ground cytoplasm are probably responsible for the fraction of microsomal particles obtained from pea seedlings by Ts'o *et al.* (23). Bayley (unpublished data) has isolated a similar fraction from pea embryos.

The cells of the embryos are also well endowed with mitochondria structurally similar to those of animal tissues (24). This agrees with the finding that wheat embryos are a good source of isolated mitochondria with biochemical activity similar to that of animal mitochondria (22). However, a considerable number of the structures resembling mitrochondria lacked cristae. Lund *et al.* (12) on the basis of studies on roots consider such structures to represent immature mitochondria. The embryo cells may contain such immature mitochondria but it is probable that at least some of the bodies lacking cristae are proplastids. Structures considered proplastids by other authors (11, 14) are essentially indistinguishable from the immature mitochondria of Lund *et al.* (12). This raises the possibility that mitochondria and plastids have a common origin, a suggestion frequently advanced on the basis of optical microscope studies (16).

The finding of both small, approximately isodiametric mitochondria and long, filamentous ones is in agreement with older optical microscope observations on plant tissues (16). However, aside from the size difference there appears to be no structural distinction between the two types of mitochondria.

Until a functional difference is demonstrated there seems little value in employing the older terms mitosome or chondriocont (16) for the filamentous mitochondria. These filamentous mitochondria have not been found in preparations of isolated mitochondria from wheat embryos (22), either because they are rare or because they are broken during cell fractionation.

The isolated embryos here studied are capable of considerable growth when supplied with only water, indicating that significant amounts of reserve materials are present. Starch of the amyloplasts is part of this material and the dense bodies in the wheat embryo probably represent stored lipids which are responsible for the reported 10% fat content of wheat germ (3). In addition it is possible that the irregular bodies represent stored proteinaceous material. The structure and density of these bodies vaguely suggests that they are formed from an accumulation of protein within the membranes of the endoplasmic reticulum. It has been suggested that protein may accumulate within the endoplasmic reticulum of animal cells (18).

The function of the prominent thick-walled bodies of the wheat embryo is unknown. These bodies are undoubtedly responsible for the variety of cytoplasmic particles observed in the phase-contrast microscope. Modilevski and Bayliss (13) in an optical microscope study of germinating wheat embryos describe similar cytoplasmic structures and report that they disappear as germination proceeds. This suggests that these bodies represent a complex form of reserve material utilized during germination. The thick-walled bodies may also be responsible for the unique oxidative fraction isolated from wheat embryos by Stern and Johnston (21). It is possible that the different forms of thick-walled bodies described represent stages of a developmental sequence: completely solid bodies (Fig. 20) through hollow bodies (Figs. 3, 17, 18) to disrupted membranes (Figs. 7, 19).

Press (20) and Lund *et al.* (12) picture structures in cells of roots which are somewhat similar to one form of the thick-walled bodies (Fig. 17). Press identifies these structures with the osmiophilic platelets described by Bowen (1) from optical microscope studies. Possibly the thick-walled bodies of the embryos represent a highly developed form of the structures in roots but identification with the controversial osmiophilic platelets (15) seems undesirable at present.

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EXPLANATION OF FIGURES

Figs. 1–4 are phase-contrast photomicrographs, Figs. 5–20 electron micrographs. All micrographs from sections. Scale lines represent 1 micron.

A, amyloplast
C, presumed chromosome
Cl, clear region of nucleolus
D, dense body
Dg, dense granule of amyloplast
E, endoplasmic reticulum
G, dense cytoplasmic granules
I, irregular-shaped body
M, mitochondrion
Ma, matrix of amyloplast
N, nucleus
Nm, nuclear membrane
Nu, nucleolus
Pd, plasmodesm
Pm, plasma membrane
Pp, possible proplastid
S, starch grain
T, thick-walled body
V, vesicle
W, cell wall

PLATE I

FIG. 1. Pea-embryo cells near the epicotyl meristem, stained with I_2–KI. ×1200
FIG. 2. Pea-embryo cells near the base of the cotyledons, stained with I_2–KI. ×1200
FIG. 3. Wheat-embryo cells near the base of the epicotyl. ×1200
FIG. 4. Wheat-embryo cells near the base of the coleorhiza. ×1200

PLATE II

FIG. 5. Low magnification view of a relatively undifferentiated pea-embryo cell showing the structures common to all cells observed. ×9000

PLATE III

FIG. 6. Low magnification view of a differentiated wheat-embryo cell remote from the meristems. In addition to the structures present in the cell of Fig. 5 the cytoplasm contains prominent thick-walled bodies and dense bodies. ×9000

PLATE IV

FIG. 7. Same as Fig. 6 except that thick-walled bodies of a different type are present in the cytoplasm. The section has not passed through the nucleus. ×9000

PLATE V

FIG. 8. Pea-embryo cell showing detail of the nucleolus, nucleus, and adjacent cytoplasm. The ridges in the nucleolus are due to compression during sectioning. ×20,000

PLATE VI

FIG. 9. Cytoplasm of a wheat-embryo cell, showing endoplasmic reticulum. ×40,000
FIG. 10. A small multilamellar body in the cytoplasm of a pea-embryo cell. ×26,500
FIG. 11. Cytoplasm of a wheat-embryo cell showing portions of the long mitochondria occasionally encountered. ×40,000

PLATE VII

FIG. 12. The cell-wall region of a wheat-embryo cell. ×40,000
FIG. 13. Typical mitochondria and irregular-shaped bodies in the cytoplasm of a wheat-embryo cell. ×33,000

PLATE VIII

FIG. 14. Amyloplasts of a pea-embryo cell. ×40,000
FIG. 15. Amyloplasts of a wheat-embryo cell. ×33,000
FIG. 16. A structure in the cytoplasm of a pea-embryo cell which may represent a proplastid. ×26,500

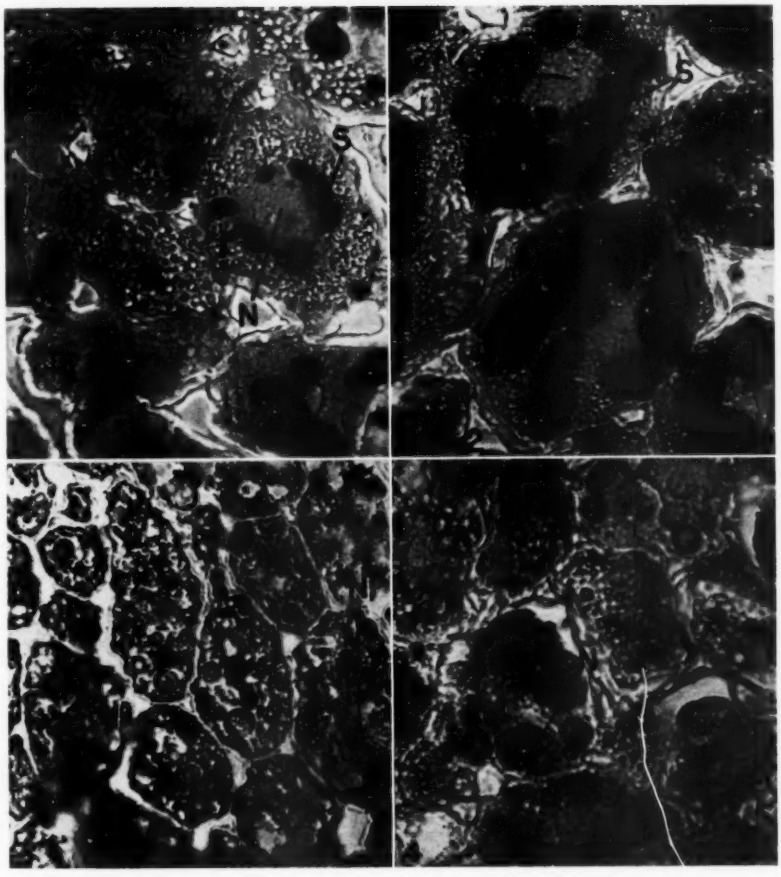
PLATE IX

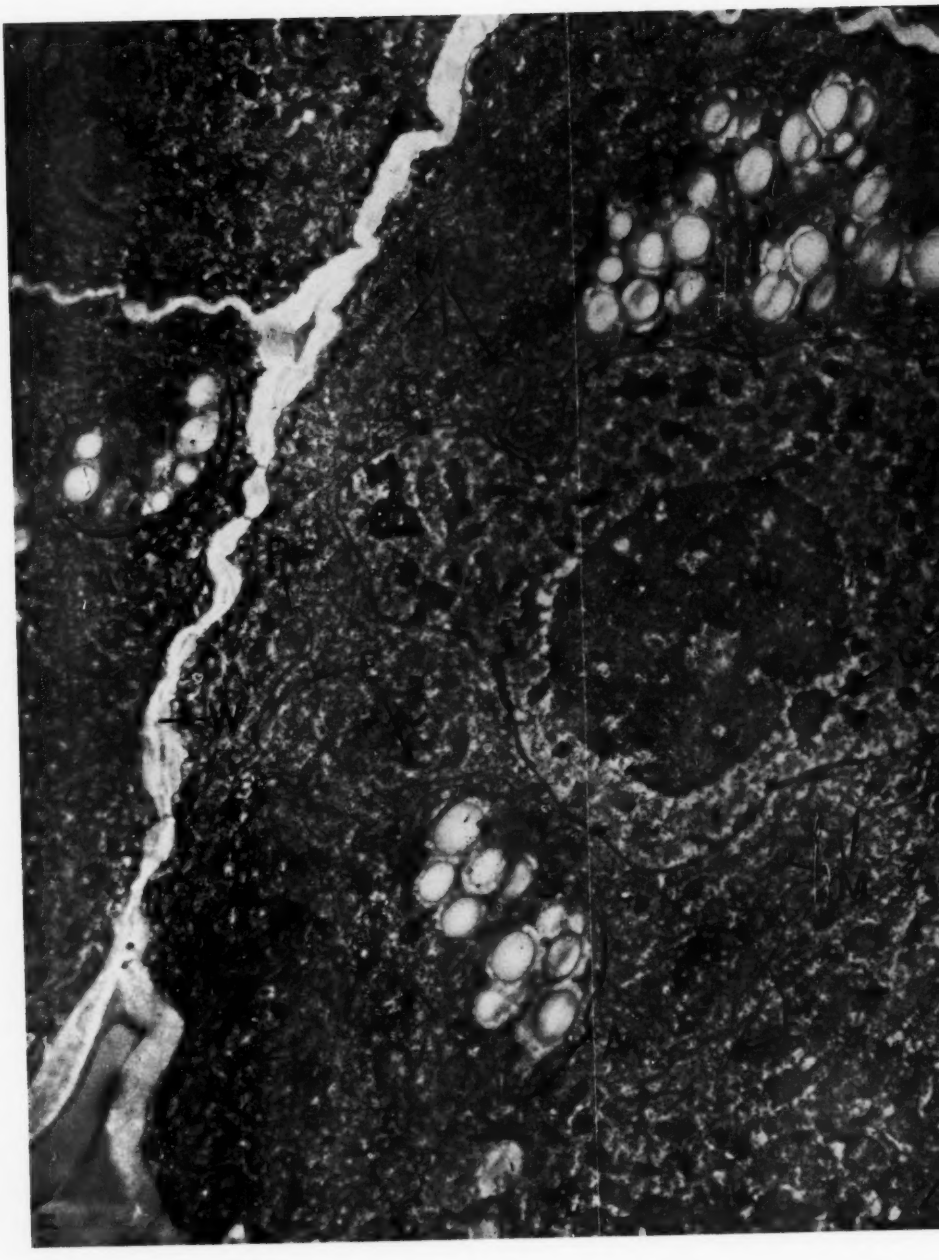
FIGS. 17–18. Two forms of thick-walled bodies in the cytoplasm of wheat-embryo cells (cf. Fig. 6). Both ×26,500

PLATE X

FIGS. 19–20. Two forms of thick-walled bodies in the cytoplasm of wheat-embryo cells (cf. Fig. 7). Fig. 19. ×33,000. Fig. 20. ×26,500

PLATE I





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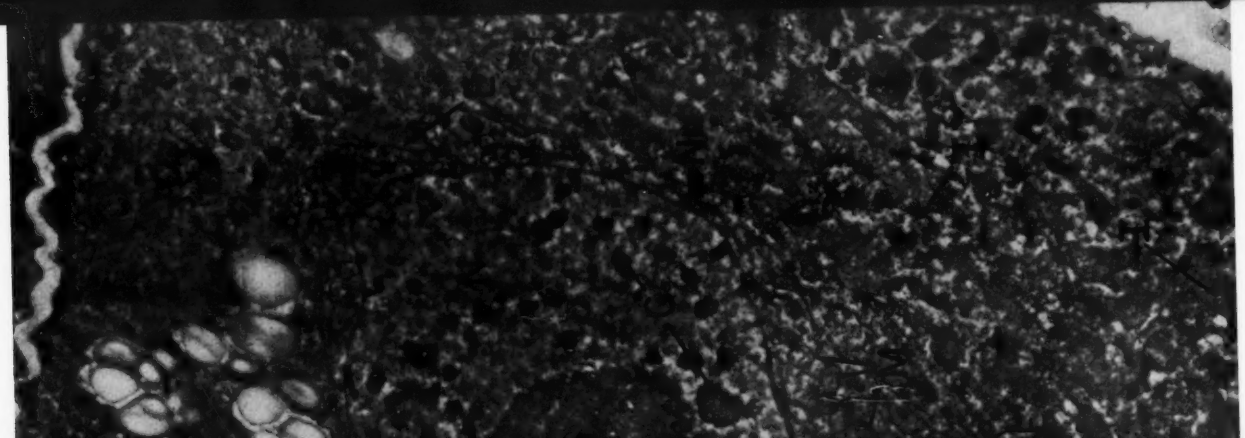

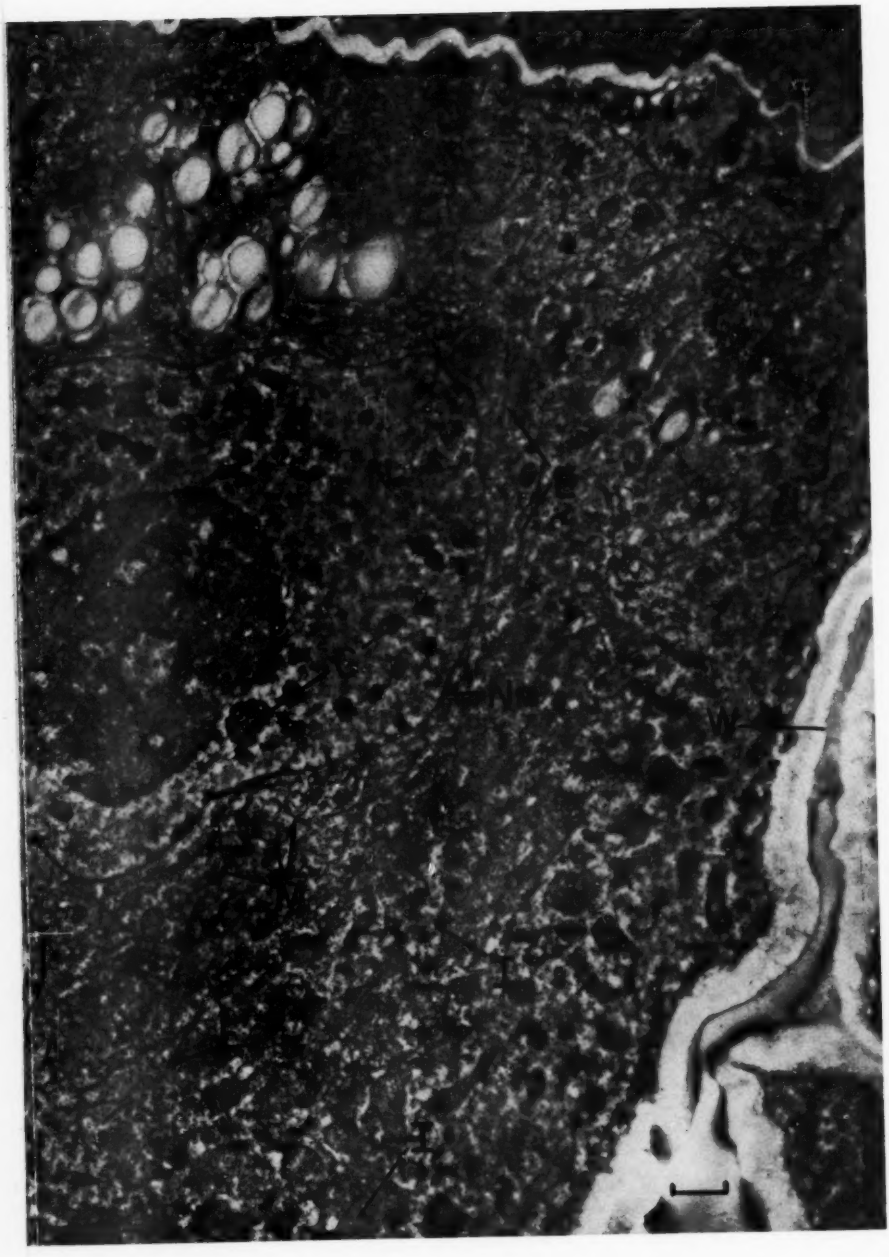

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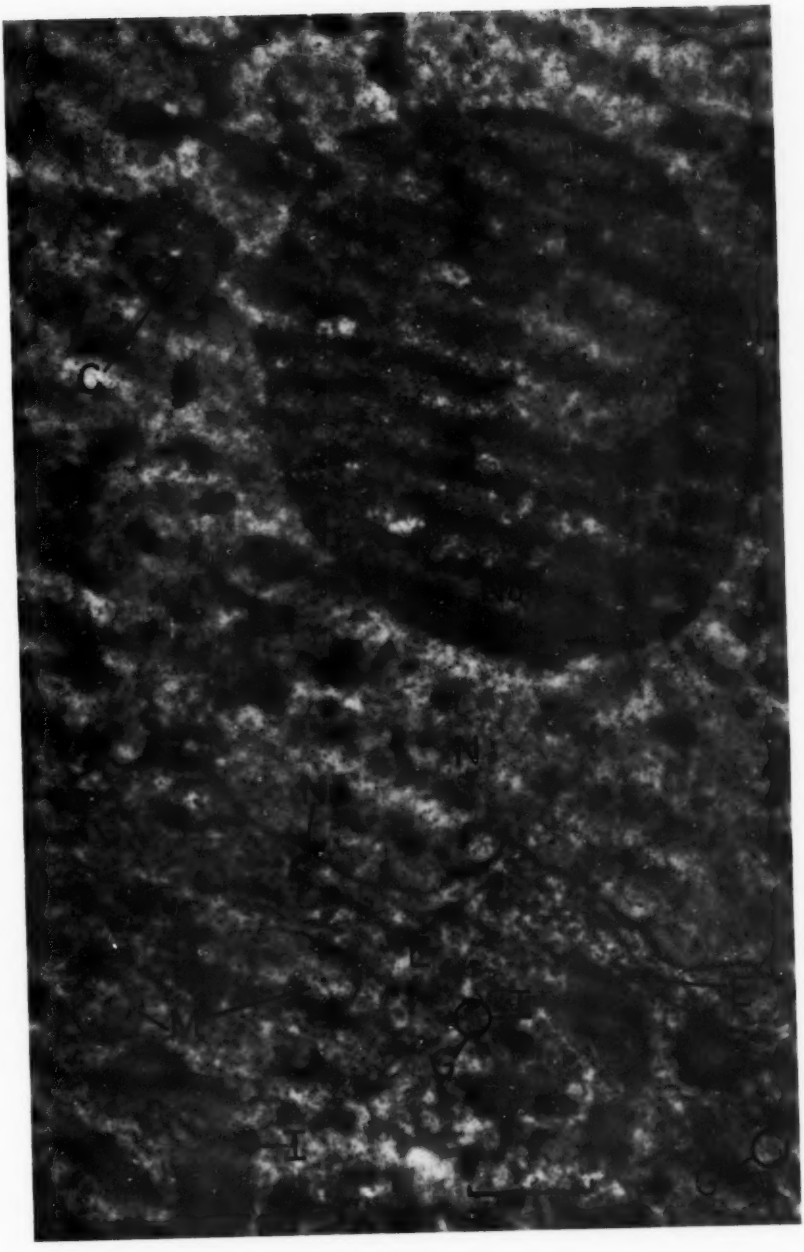

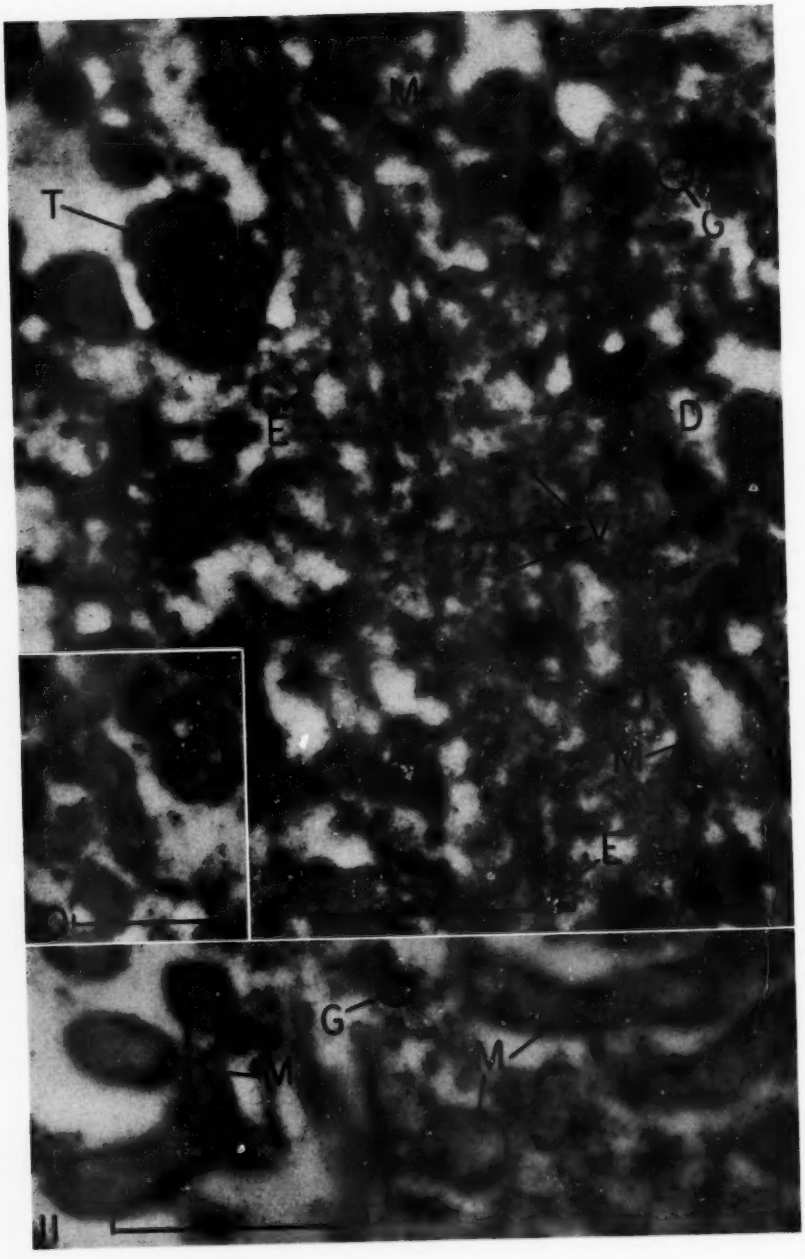

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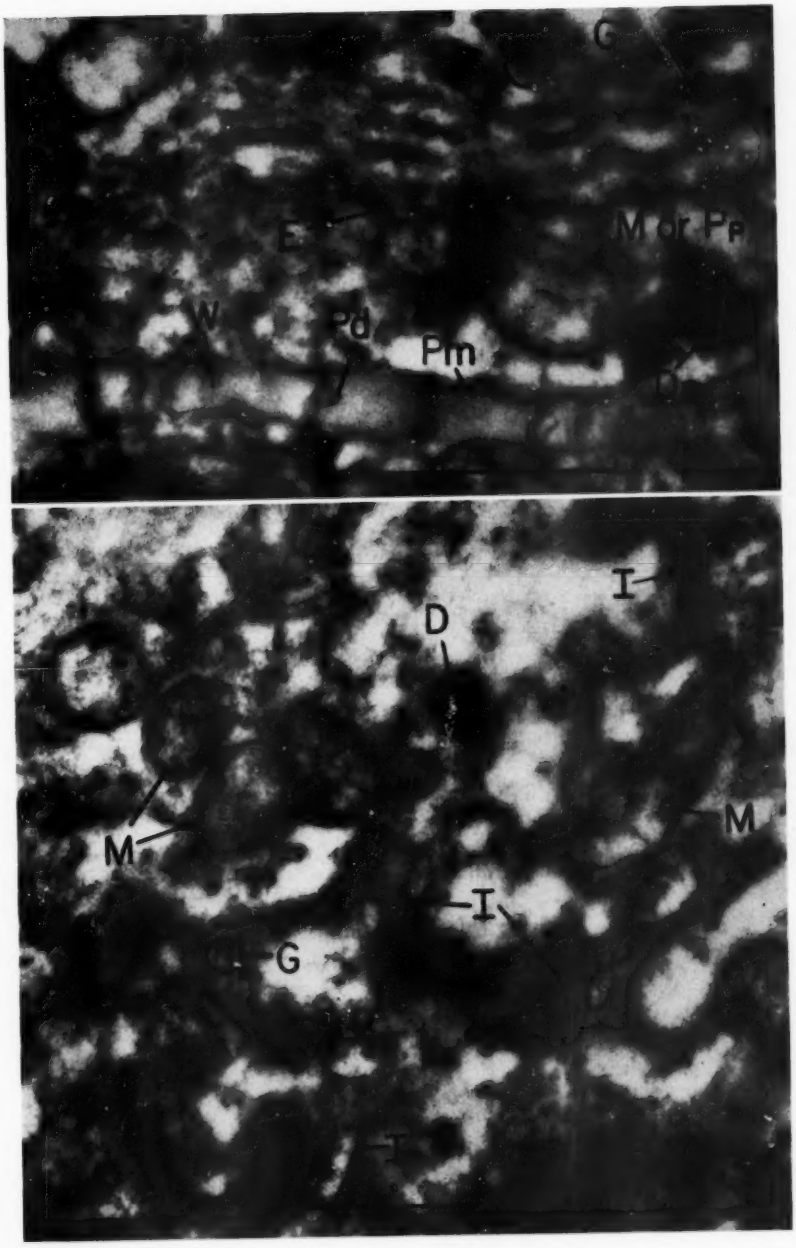

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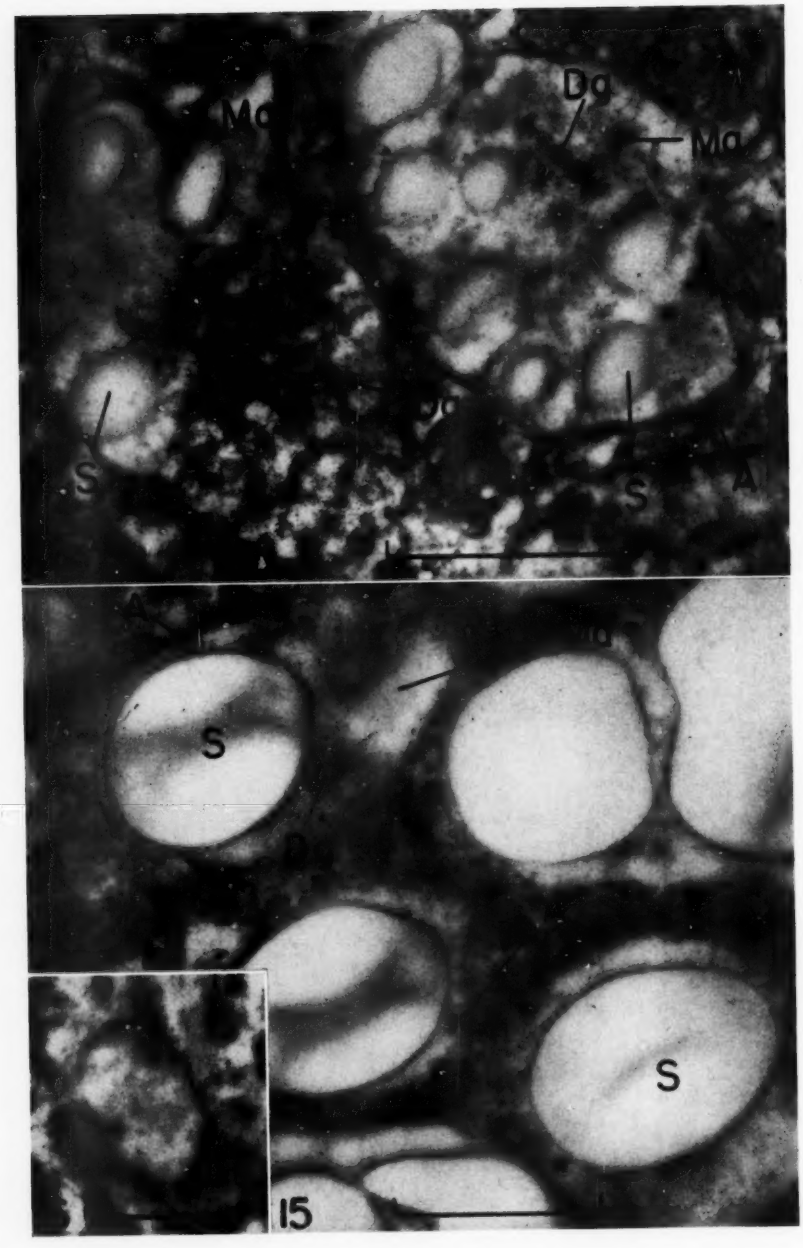

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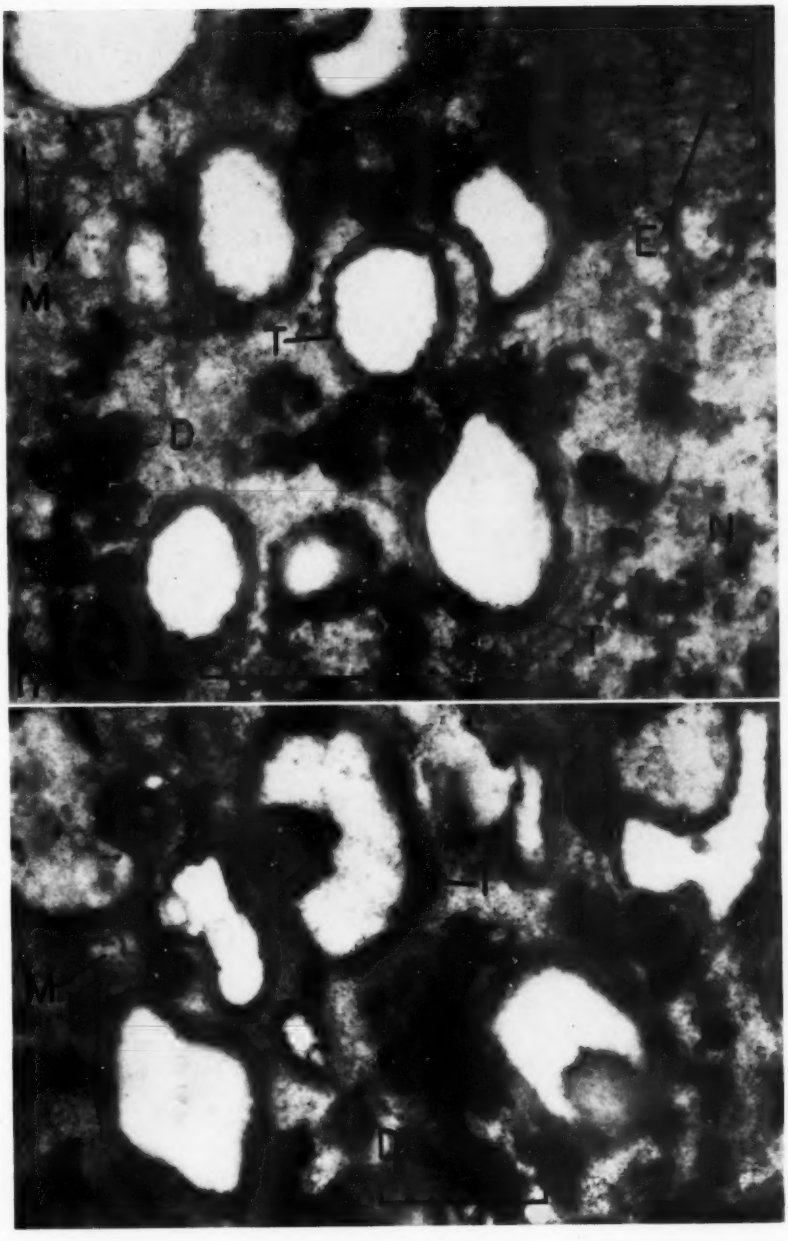

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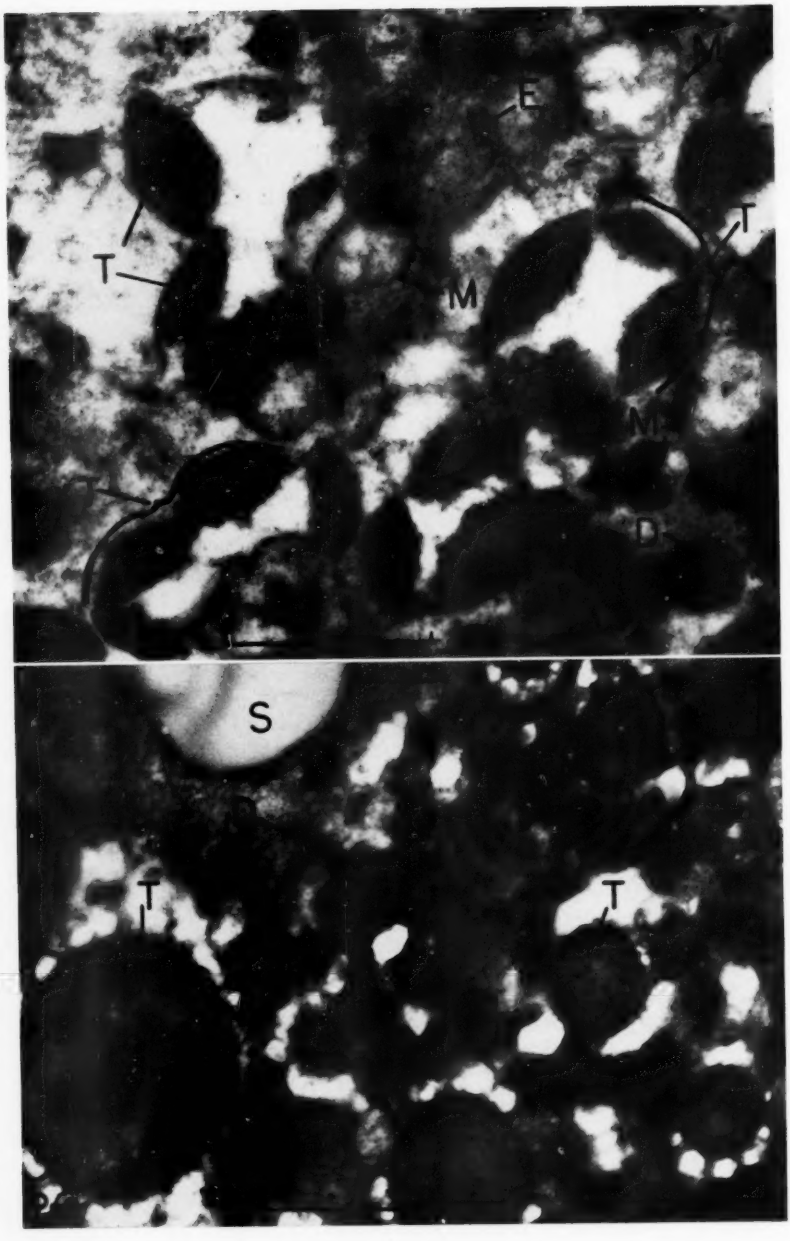
PLATE X



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ACHILLEA MILLEFOLIUM COMPLEX IN CANADA AND PORTIONS OF THE UNITED STATES[1]

GERALD A. MULLIGAN[2] AND I. JOHN BASSETT[2]

Abstract

The chromosome numbers of plants of the *Achillea millefolium* complex from 27 locations in North America are reported. The levels of polyploidy found are correlated with diameters of the pollen grains. Measurements of pollen grains from more than 300 herbarium specimens are used as an indication of the occurrence of tetraploid and hexaploid species of *Achillea* in Canada. The results of some crossing experiments are also reported. It is concluded that the common *Achillea* of North America is tetraploid and is the native *A. lanulosa* Nutt. It extends from the Pacific to the Atlantic coasts, as far south as California in the west and Pennsylvania in the east, and to northern Canada and Alaska. Another native species, hexaploid *Achillea borealis* Bong., extends along the Pacific coast from southern California to the Aleutian Islands and along the northern shorelines of Alaska and Canada. A Eurasiatic hexaploid, *A. millefolium* L. s. str., has been sparingly introduced into Newfoundland, Prince Edward Island, Nova Scotia, and perhaps elsewhere along the Atlantic seaboard. In eastern Canada a male-sterile purple-flowered hexaploid also occurs. This hexaploid escapes from cultivation and forms localized patches. It undoubtedly has been selected from the Eurasiatic *A. millefolium* L. s str.

Introduction

The *Achillea millefolium* complex is represented in Eurasia by many species, of which *A. millefolium* L. s. str., $2n = 54$, is the most widespread. In North America, however, this complex, as we know it at present, is represented by only two native species, *A. lanulosa* Nutt., $2n = 36$, and *A. borealis* Bong., $2n = 54$. Clausen, Keck, and Hiesey (1, 2, 3), Lawrence (7), and Turesson (8, 9) found that plants of yarrow from many locations in California, Oregon, Washington, and a few locations in British Columbia, Idaho, Alberta, Montana, North Dakota, New York, Massachusetts, Vermont, and New Brunswick had 18 pairs of chromosomes but plants from along the coastlines of California, Oregon, Washington, and Alaska had 27 pairs of chromosomes. They concluded that western North American yarrow plants constitute indigenous species and decided that the tetraploids were *A. lanulosa* Nutt., and the hexaploids *A. borealis* Bong. Ehrendorfer (4) using chromosome counts and a correlation of chromosome counts and pollen grain size stated that tetraploid *A. lanulosa* occurs widely in the western two thirds of the United States and Canada and north to Yukon and Alaska in the extreme western part of this continent. He also stated that hexaploid *A. borealis* occurs almost continuously along the Pacific coast from southern California to the Aleutian Islands. As this paper was being prepared for publication, Ehrle (5) published chromosome counts of plants of the *Achillea millefolium* complex from 17 locations in Pennsylvania. All plants counted had a somatic

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chromosome number of $2n = 36$ and Ehrle therefore concluded that the Pennsylvania plants were native *A. lanulosa*, rather than *A. millefolium* L. s. str.

It has been suspected that the Eurasiatic hexaploid *A. millefolium* L. s. str. was also present on this continent, especially in eastern North America. However, very little material of the *A. millefolium* complex from eastern Canada and the northeastern United States has been examined cytologically and the small amount studied has had the chromosome number of native *A. lanulosa*. It has, so far, been impossible to find good morphological characters, other than the size of pollen grains, to permit the separation of plants at the two chromosome levels. Although some ecotypes at both polyploid levels can be recognized morphologically, the number of characters that vary in both *A. lanulosa* and *A. borealis* is so great and the intergradation so complete that many plants of the former species are morphologically indistinguishable from many plants of the latter species. Furthermore, it is virtually impossible to distinguish many plants of both native species from the typical *A. millefolium* of Eurasia. However, Hiesey and Nobs (6) found that the genetic systems of the two hexaploids, *A. borealis* Bong., and *A. millefolium* L. s. str., have diverged so far that crosses between them do not yield viable seed.

Because chromosome numbers are a critical basis for the separation of tetraploid *A. lanulosa*, hexaploid *A. borealis*, and the Eurasiatic hexaploid *A. millefolium* L. s. str., the need for further information on the chromosome numbers of North American material, especially on plants from eastern Canada and the United States, is apparent. Therefore, most of the plants examined cytologically were collected from this area. In addition, chromosome numbers were correlated with size of pollen grains, and pollen taken from herbarium specimens collected in Canada and Alaska was measured. Finally, intercrosses were attempted between *A. lanulosa*, *A. borealis*, and *A. millefolium* L. s. str.

Materials and Methods

Plants were grown in a greenhouse from clones collected in the wild, and in a few instances from seed. Root tips were taken from potted plants, placed in water, and then exposed to a temperature of 2°–4° C for 2 to 3 hours. They were fixed in Nawaschin solution, embedded in paraffin, and sectioned in the usual manner. The slides were stained in crystal violet. All plants counted were transferred to nursery plots for observation. Voucher specimens were collected in the nursery and are preserved in the Canada Department of Agriculture Herbarium, Ottawa.

Pollen grains, removed from open flowers of dried specimens from the herbaria of the Department of Agriculture and National Museum at Ottawa, were examined in glycerine jelly on glass slides. The polar views, from spine to spine, of 10 well-formed pollen grains were measured.

TABLE I

Chromosome numbers and pollen grain size of 32 collections of the *Achillea millefolium* complex

Species and authors' chromosome counts	Source of seed, collector, and authors' collection numbers	Mean outside diameter of 10 pollen grains, μ
Achillea lanulosa, $2n = 36$	British Columbia	
	Dawson Creek, A. J. Breitung (2320)	29.4 (29.3 to 30.9)
	Alberta	
	Foremost, D. R. Lindsay (2160)	28.9 (26.0 to 30.9)
	Utah	
	Marlboro, W. B. Johnson (2316)	28.5 (26.0 to 30.9)
	Ontario	
	Manitoulin Is., I. J. Bassett (2307)	27.7 (26.0 to 30.9)
	Bloomfield, I. J. B. (2308)	29.8 (26.0 to 30.9)
	16 mi. SE. Kemptville, G. A. Mulligan (2306)	29.6 (26.0 to 30.9)
	Sand Point, C. Frankton (2364)	26.9 (26.0 to 27.7)
	4 mi. N. Kemptville, G. A. M. (2323)	26.8 (26.0 to 29.3)
	Quebec	
	Quebec, A. Hamel	No voucher specimen
	Ste. Anne de la Pocatiere, A. H. (2326)	29.5 (27.7 to 30.9)
	New York	
	Oswego, D. R. Sampson (2312)	28.0 (26.0 to 29.3)
	Carlisle, D. R. S. (2314)	28.5 (27.7 to 30.9)
	New Jersey	
	Ridgewood, T. Parsons (2301)	27.4 (26.0 to 29.3)
	Massachusetts	
	Amherst, W. B. J. (2302)	27.2 (26.0 to 30.9)
	Rhode Island	
	East Greenwich, D. R. S. (2324)	29.6 (27.7 to 30.9)
	Maine	
	Dover-Foxcroft, F. C. Bent	No voucher specimen
	Katadin Field Campground, J. P. Revel (2317)	28.2 (26.0 to 29.3)
	Sourduabunk Campground, J. P. R. (2315)	29.0 (26.0 to 30.9)
	New Brunswick	
	St. Stephen, F. C. B. (2313)	28.8 (27.7 to 30.9)
	Woodstock, E. G. Anderson (2321)	28.3 (26.0 to 30.9)
	Nova Scotia	
	Margaretsville, F. C. B. (2300)	28.2 (26.0 to 30.9)
	Cape Forchu, E. C. Smith (2309)	27.0 (26.0 to 29.3)
Achillea millefolium, $2n = 54$	Ontario	
	Morrisburg, G. A. M. (2295)	No pollen
	Prince Edward Island	
	Charlottetown (white fls.), E. G. A. (2303)	No pollen
	Charlottetown (pink fls.), E. G. A. (2311)	31.7 (30.9 to 32.5)
	Newfoundland	
	St. John's (white fls.), E. G. A. (2319)	31.7 (30.9 to 32.5)
	St. John's (pink fls.), E. G. A. (2299)	32.0 (30.9 to 32.5)

TABLE I—*Cont'd*

Chromosome numbers and pollen grain size of 32 collections of the *Achillea millefolium* complex

Species and authors' chromosome counts	Source of seed, collector, and authors' collection numbers	Mean outside diameter of 10 pollen grains, μ
Achillea millefolium, $2n = 54$ (*Cont'd*)	Germany Eberswald Botanic Garden (2310)	31.7 (30.9 to 32.5)
	Cultivated Hillier & Sons Nursery, England (2318)	No pollen
	Hillier & Sons Nursery, England (2322)	No pollen
Achillea borealis ssp. *arenicola* ($2n = 54$ by Clausen, Keck, and Hiesey)	Received from J. Clausen and originally collected along Bodego coast, Sonoma Co., California (2297)	32.5 (30.9 to 34.1)
Achillea borealis ssp. *gigantea* ($2n = 54$ by Clausen, Keck, and Hiesey)	Received from J. Clausen and originally collected near Selma, Fresno Co., California (2298)	32.3 (30.9 to 34.1)

Results

Chromosome numbers of 32 collections of plants in the *Achillea millefolium* complex are included in Table I. Sources of plants from which chromosome counts were made, by the present workers and previous workers, are shown in Fig. 1. Twenty-four lots of material studied by the authors from British Columbia, Alberta, Utah, New York, New Jersey, Massachusetts, Rhode

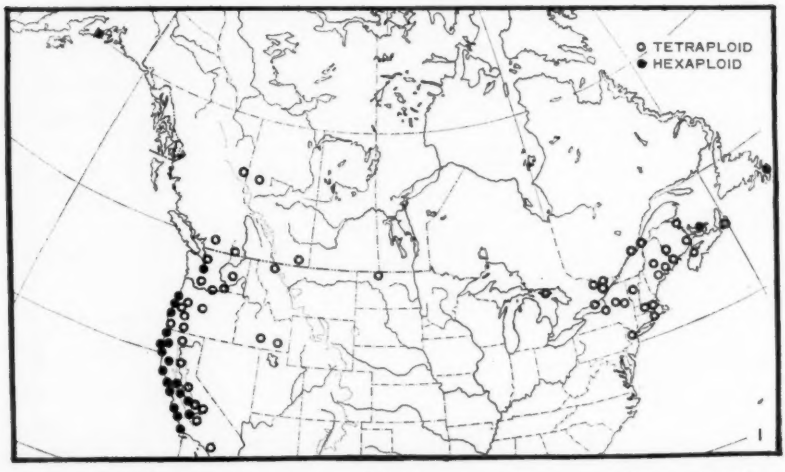


FIG. 1. Distribution of plants from which chromosome counts in *Achillea millefolium* complex were obtained.

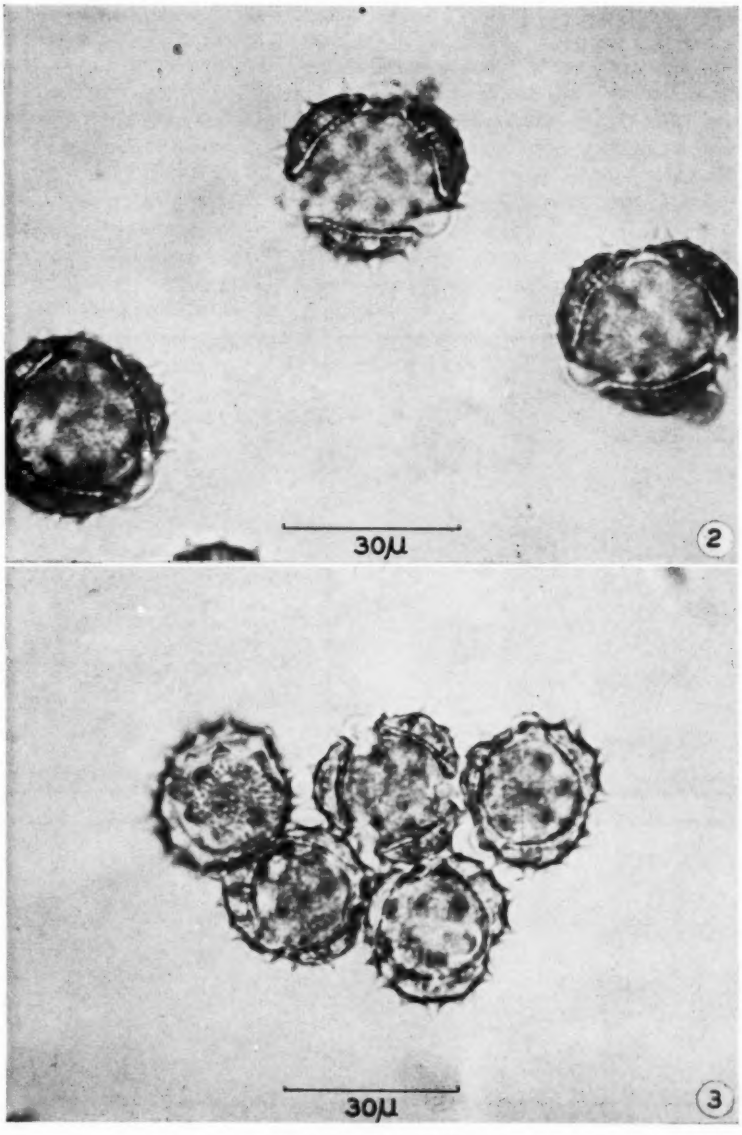
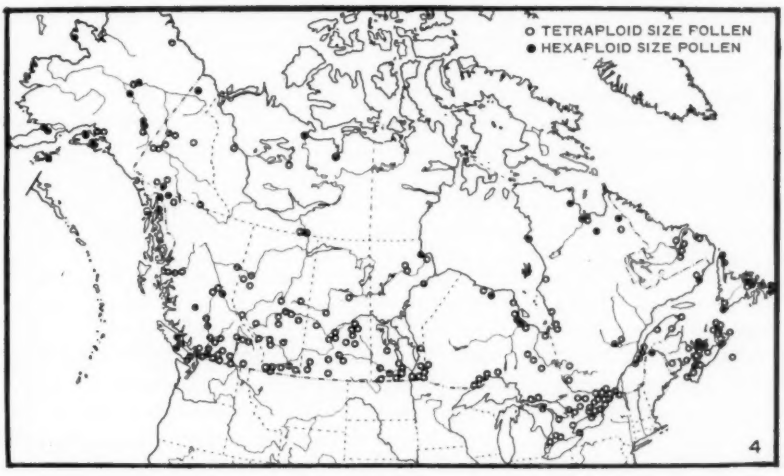


FIG. 2. Pollen grains from hexaploid *A. borealis*.
FIG. 3. Pollen grains from tetraploid *A. lanulosa*.

Island, Maine, Ontario, Quebec, New Brunswick, and Nova Scotia were tetraploid, $2n = 36$. Five collections from Ontario, Prince Edward Island, and Newfoundland were hexaploid, $2n = 54$; the remaining three collections, from European sources, were also hexaploid.

Pollen grains from over 300 herbarium specimens were measured and classified as "tetraploid" or "hexaploid". The mean outside diameter of 10 pollen grains from each collection was calculated and a relationship was found to exist between size of pollen grains and the number of chromosomes. The larger size of the hexaploid pollen grains can be seen by comparing the photomicrographs of hexaploid and tetraploid pollen grains in Figs. 2 and 3, respectively. Specimens with a mean pollen grain diameter of less than 31.5 μ were considered to be tetraploid and specimens with a mean pollen grain diameter of more than 31.5 μ were considered hexaploid. The locations of representative specimens are plotted in Fig. 4. Herbarium specimens



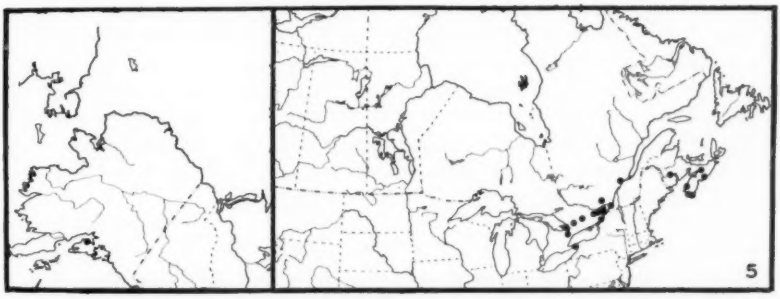


FIG. 4. Distribution of herbarium specimens with tetraploid and hexaploid size pollen grains.

FIG. 5. Distribution of herbarium specimens of purple-flowered male-sterile *Achillea millefolium* L. s. str.

with tetraploid size pollen grains were collected from all 10 Canadian provinces, Yukon, Northwest Territories, and Alaska. Herbarium specimens with hexaploid size pollen grains were collected along the coastlines of British Columbia, Alaska, Yukon, Northwest Territories, Hudson and James bays, northern Quebec, Prince Edward Island, Nova Scotia, and Newfoundland. Herbarium specimens of a dark purple-flowered plant collected mostly in eastern Ontario, southern Quebec, New Brunswick, and Nova Scotia had no pollen. These specimens are mapped in Fig. 5.

Tetraploid and hexaploid plants from 15 of the collections listed in Table I were bagged before they flowered. Except on a few inflorescences, where several viable seeds were formed, no viable seeds were obtained. This indicated that the plants studied were almost completely self-sterile. Reciprocal crosses between tetraploid plants from Cape Forchu, N.S. (2309), and Dawson Creek, B.C. (2320), yielded several hundred well-formed seeds. These seeds germinated readily and vigorous plants were established. There are apparently no genetic barriers between eastern and western tetraploids. Reciprocal crosses between Newfoundland hexaploid (2319) and hexaploid *A. borealis* (2297) from California yielded no viable seed. Further crosses between hexaploids, Newfoundland (2319) × cultivated (2322) and Newfoundland (2319) × Newfoundland (2299), yielded an abundance of viable seed. The genetic incompatibility of the Newfoundland hexaploids and *A. borealis* and also the genetic compatibility of the Newfoundland plants and cultivated hexaploid, derived from *A. millefolium* L. s. str., indicates that some, at least, of the eastern hexaploids are introduced *A. millefolium* of Eurasia.

Discussion

Although the general opinion has been that eastern North American material of the yarrow complex was introduced *A. millefolium* L. s. str. with 54 somatic chromosomes, chromosome counts determined on 21 lots of material from Ontario, Quebec, New York, New Jersey, Massachusetts, Rhode Island, Maine, New Brunswick, and Nova Scotia were all $2n = 36$. This chromosome number is the same as that of western *A. lanulosa*. The genetic compatibility of eastern and western tetraploid plants and the continuous distribution of tetraploids across the continent indicates that eastern tetraploids are native *A. lanulosa*. It is evident from Figs. 1 and 4 that the widespread yarrow of North America is *A. lanulosa*, found from the Pacific to the Atlantic coasts, from California in the west and Pennsylvania in the east to the northern shores of Alaska, Yukon, Northwest Territories, Quebec, Labrador, and Newfoundland.

Figures 1 and 4 also show that hexaploid plants occur along the Pacific coast from California to the Aleutian Islands, across the northern coasts of Alaska, Yukon, Northwest Territories, Ontario, and Quebec, to Prince Edward Island, Nova Scotia, and Newfoundland. Our experimental crosses indicate that the clones collected at St. John's, Newfoundland, are introduced *A. millefolium* L. s. str. The two hexaploid clones from Prince Edward

Island were collected along railway tracks leading to the end of a wharf at Charlottetown. This suggests that these plants were also introduced from Europe. It is therefore probable that most of the hexaploid plants in Prince Edward Island, Nova Scotia, and Newfoundland, and perhaps eastern Quebec, are *A. millefolium* L. s. str. The hexaploids along the Pacific coast of North America are known to be *A. borealis*, and from our pollen data it appears that hexaploids also occur along the northern shorelines of Alaska and Canada. The label data for many of these northern specimens leave little doubt that the plants were collected from native habitats. It therefore appears that native *A. borealis* occurs not only along the Pacific coast from southern California to the Aleutian Islands but also across the northern shorelines of Alaska, Yukon, Northwest Territories, Ontario, and Quebec.

The dark purple-flowered hexaploid collected along a roadside near Morrisburg, Ontario, is morphologically similar to plants, lacking pollen, received from an English nursery under the names *A. millefolium* 'Cerise Queen' and *A. millefolium* 'Fire King'. Both these cultivated varieties are hexaploid and undoubtedly have been selected from Eurasiatic *A. millefolium* L. s. str. The purple-flowered plant frequently escapes from cultivation in eastern Canada (see Fig. 5). It usually forms small localized patches, suggesting that most if not all the spread is by underground rootstocks. Rarely are plants seen growing very far from the obvious source of the infestation.

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CHROMOSOME NUMBERS OF CANADIAN WEEDS. II[1]

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Abstract

The chromosome numbers of 38 Canadian weeds are reported and the significance of some of these numbers is discussed. Counts obtained on material of *Iva xanthifolia* L., $2n = 36$, *Erysimum hieraciifolium* L., $2n = 48$, and *Nepeta cataria* L., $2n = 34$, differ from those obtained by previous workers. The chromosome numbers of the following species have not previously been reported: *Rumex transitorius* Rech. f., $2n = 20$; *R. triangulivalvis* (Danser) Rech. f., $2n = 20$; *R. verticillatus* L., $2n = 60$.

Introduction

A study of chromosome numbers of Canadian weeds was initiated at the Botany and Plant Pathology Laboratory, Ottawa, in 1953 and the first list of counts was published in 1957 (Mulligan (63)). As stated in the first publication of this series, chromosome numbers are an aid to the taxonomic studies of weeds and permit a consideration of the relationships of ploidy to aggressiveness, life duration, and habitat. They also provide fundamental information on the variability or stability of different weeds. This second paper lists chromosome numbers of 38 taxa from Canada and discusses the significance of some of these.

Material and Methods

Mature seeds were collected from weeds growing in their natural habitats or from herbarium sheets. In a few instances, live material was transferred from the field to the greenhouse. The seeds were sown in the greenhouse and five plants from each lot were established. Root tips were taken from each plant, placed in water, and then exposed to a temperature of 2°–4° C for 2 to 3 hours. They were then fixed in Randolph's Nawaschin solution, embedded in paraffin, and sectioned in the usual manner. The prepared slides were stained in crystal violet. Specimens of the plants from which chromosome counts were made were collected and placed in the herbarium of the Botany and Plant Pathology Laboratory, Department of Agriculture, Ottawa (DAO). All drawings were made with the aid of a camera lucida.

Results

A complete list of the weeds studied, and their chromosome numbers, is given in Table I.

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TABLE I

Chromosome numbers of 38 Canadian weeds and references to previous counts

Species counted	Author's count		Source of material, collector, and author's collection number	Previous counts		References to previous counts
	n	2*n*		*n*	2*n*	
Caryophyllaceae						
Saponaria officinalis L.		28	Ottawa, Ont., C. Frankton (2284)	14		(32) Eur.
					28	(10, 21, 11) Eur.
Silene noctiflora L.		24	Ottawa, C. F. (1487)	12		(80, 9) Eur.
					24	(32, 57, 11) Eur.
Chenopodiaceae						
Chenopodium glaucum L. var. *glaucum*		18	Goose Bay, Labrador, J. M. Gillett (2290); Collingwood, Ont., I. J. Bassett (2289)		18	(100) Eur.
glaucum L. var. *salinum* (Standley) Boivin		18	Brandon, Man., G. A. Stevenson (2288)		18	(53) N. Amer.?
Compositae						
Chrysanthemum leucanthemum L. s. str.	9		Morrisburg, Ont., G. A. M. (2198); Metcalfe, Ont., G.A.M. (2199); 16 mi. SSE. Kemptville, Ont., G.A.M. (2197); Ottawa, C.F. (2196); Sand Point, Ont., C.F. (2208)		18	(74, 19, 13) Eur.; (60, 64) U.S.A.; (64) Can.
		18	St. John's, Nfld., I.J.B. (2373); Goose Bay, Labrador, J.M.G. (2252); Charlottetown, P.E.I., E. G. Anderson (2374); Annapolis Royal, N.S., H.A. Senn (2261); Moncton, N.B., H.A.S. (2257); Belledune Point, N.B., H.A.S. (2263); Bar Harbour, Maine, H.A.S. (2262); Lac au Saumon, Que., H.A.S. (2256); St. Fabien, Que., H.A.S. (2259); Ste.-Angele-de-Merici, Que., H.A.S. (2258); Chicoutimi, Que., E.G.A. (2264); Laterriere, Que., E.G.A. (2265); St. Pierre de Montmagny, Que., H.A.S. (2266); Batiscan, Que., H.A.S. (2375); St. Alexis, Que., H.A.S. (2376); Yamachiche, Que., H.A.S. (2255); St. Canut, Que., H.A.S. (2377); Grenville Station, Ont., J. W. MacRae (2378); Monckland Station, Ont., G.A.M. (2269); Almonte, Ont., W. T. James (no voucher spec.); Sydenham, Ont., W. M. Scott (2251); Midland, Ont., I.J.B. (2116); Bloomfield, Ont., I.J.B. (2379); Manitoulin Island, Ont., I.J.B. (2380); Creston, B.C., G.A.M. (2381); Langley Prairie, B.C., G.A.M. (2271)			
leucanthemum L. vars.		36	Tidehead, N.B., H.A.S. (2254); Lauzon, Que., H.A.S. (2260); Lennoxville, Que., E.G.A. (2253); Batiscan, Que., H.A.S. (2388); Bot. Garden, Besançon, France, (2272); Bot. Garden, Moscow, U.S.S.R. (2268)	18		(89, 90, 84) Asia
					36	(19, 56, 13, 64) Eur.; (16) U.S.A.; (64) Can.
		54	Bot. Garden, Porto, Portugal as *Leucanthemum vulgare* Hill ssp. *pallens* DC. (2150); Bot. Garden, Porto, Portugal as *Leucanthemum vulgare* Hill ssp. *crassifolium* Hoff. & Link. (2151)		54	(19, 13, 64) Eur.

TABLE I (*Continued*)

Chromosome numbers of 38 Canadian weeds and references to previous counts

Species counted	Author's count n	Author's count $2n$	Source of material, collector, and author's collection number	Previous counts n	Previous counts $2n$	References to previous counts
Crepis capillaris (L.) Wallr.		6	Haney, B.C., D. Faris (2120)		6	(76) Eur.; many other counts of mostly $n = 3$ or $2n = 6$, Eur., Asia, and U.S.A.
tectorum L.		8	Morden, Man., W. J. Breakey (1514)	4	8	(43) Eur.; many other counts of mostly $n = 4$ or $2n = 8$, Eur. and U.S.A.
Iva xanthifolia Nutt.		36	Swift Current, Sask., K. F. Best (2279)	14		(71) Eur.
Matricaria maritima L. var. *agrestis* (Knaf.) Wilmott (*Matricaria inodora* L.)		18	Cape Forchu, N.S., E. C. Smith (2340); Lowe's Landing, N.S., E.C.S. (2349); Truro, N.S., E.G.A. (2357); Northwest Cove, N.S., E.C.S. (no voucher spec.); Linden, N.S., E.C.S. (no voucher spec.); Notre Dame, N.B., I.J.B. (2341); St. John, N.B., G.A.M. (2342); Ste. Anne de la Pocatiere, Que., E.G.A. (2365)	9		(35, 27) Eur.
		18+1B	Kinkora, P.E.I., I.J.B. (2350); Charlottetown, P.E.I., I.J.B. (2354)			
		18+2B	Kinkora, P.E.I., I.J.B. (2351)			
		36	Markinch, Sask., C.F. (no voucher spec.); Balgonie, Sask., J. W. Hudson (2344); Balgonie, Sask., C.F. (no voucher spec.); Kandahar, Sask., J. W. Selleck (2343); Stockholm, Sask., J.W.S. (2339)	18		(30) Eur.
					36	(77, 96) Eur.
Rudbeckia serotina Nutt.		38+1B	Kemptville, Ont., G.A.M. (2283)	19		(4) Eur.
Senecio jacobaea L.		40	Abbotsford, B.C., G.A.M. (2282)		32	(12) Eur.
				20		(1) Eur.
					40	(12) Eur.
Taraxacum officinale Weber		24	Sault Ste. Marie, Ont., K. K. Bragg (1491)		24	(26) Eur.; many other counts of either $n = 12$ or $2n = 24$, Eur. and U.S.A.
Cruciferae						
Barbarea vulgaris R. Br.		16	Ottawa, I.J.B. (2286)	8		(61) Eur.; (86) U.S.A.
					16	(59, 56) Eur.
Brassica campestris L.		20	Bathurst, N.B., G.A.M. (1482)	10		(95) Asia; many other counts of either $n = 10$ or $2n = 20$, Eur., Asia, and U.S.A.
juncea (L.) Coss.		36	Lethbridge, Alta., D.R. Lindsay (1483)		36	(2) Asia; many other counts of either $n = 18$ or $2n = 36$, Eur., Asia, and U.S.A.
Diplotaxis tenuifolia (L.) DC.		22	Harrow, Ont., G.A.M. (2273)	7		(41) Eur.
				11		(36) S. Amer.; (58) Eur.
					22	(59, 3, 58) Eur.
Erysimum hieraciifolium L.		48	Mayerville, Ont., K.K.B. (2137)		32	(39, 56) Eur.
Geraniaceae						
Geranium molle L.		26	Chilliwack, B.C., D. Faris (2285)	13		(99) Eur.
					26	(23, 99, 56) Eur.

TABLE I (*Continued*)

Chromosome numbers of 38 Canadian weeds and references to previous counts

Species counted	Author's count n	Author's count $2n$	Source of material, collector, and author's collection number	Previous counts n	Previous counts $2n$	References to previous counts
Labiatae						
Galeopsis tetrahit L.		32	Vernon, B.C., G.A.M. (2149)	16		(65) Eur.
					32	(65, 56) Eur.
Nepeta cataria L.		34	Brackley Beach, P.E.I., D. Erskine (2383); Kentville, N.S., E.G.A. (2382); Ottawa, G.A.M. (2384); Kelowna, B.C., G.A.M. (2153)	16		(14) Eur.
				18		(88) Asia
Plantaginaceae						
Plantago major L.		12	Ottawa, G.A.M. (1445)	6		(20) Eur.; many other counts of either $n = 6$ or $2n = 12$, Eur. and U.S.A.
				12		(38, 91) Asia
					24	(38, 37, 91) Asia
Polygonaceae						
Rumex acetosella L.		42	Ottawa, C.F. (1456); Bells Corners, Ont., C.F. (2367); Murraydale, Sask., R. T. Coupland (2366); Penticton, B.C., G.A.M. (2243); Hatzic, B.C., G.A.M. (2245); Vancouver, B.C., G.A.M. (2244)		14	(50) Eur.
				14		(61) Eur.
					28	(48, 49, 50, 51, 52) Eur.
				21		(44) Asia; many other counts of either $n = 21$ or $2n = 42$, Eur., Asia, U.S.A., and Can.
					56	(50, 52) Eur.
longifolius DC.		60	Bedford, Que., C.F. (2368); Temagimi, Ont., I.J.B. (2241)	30		(45) Eur.
					60	(51, 56) Eur.
				40		(45) Asia
maritimus L. var. *fueginus* (Phil.) Dusen		40	Brackley Beach, P.E.I., D. Erskine (2184)		40	(53) N. Amer.?
obtusifolius L.		40	Langley Prairie, B.C., L. Jenkins (2242)	20		(45) Eur.; (45, 44) Asia
					40	(51, 56) Eur.
transitorius Rech. f.		20	Prince Rupert, B.C., J. A. Calder (2202); Esquimalt, Vancouver Island, B.C., W.E. Molyneux (2201)	None		
triangulivalvis (Danser) Rech. f.		20	Charlottetown, P.E.I., D. Erskine (2200); Ottawa, G.A.M. (1477); Fort Simpson, B.C., W. J. Cody (2370)	None		
verticillatus L.		60	Ottawa, C.F. (2371)	None		
Ranunculaceae						
Ranunculus acris L.		14	Ottawa, G.A.M. (2134); Noelville, Ont., I.J.B. (2133)		12	(87) Eur.; (46) Asia
				7	14	(81) Eur.; many other counts of either $n = 7$ or $2n = 14$, Eur., Asia, and U.S.A.
				8		(61) Eur.
					16	(33) Eur.
					28	(81, 47, 72) Eur.
					56	(72) Eur.
Rosaceae						
Potentilla argentea L.		28	Upton, Quebec, C.F. (2155)	7		(66, 67) Eur.
					14	(67, 68, 69, 70, 28) Eur.
		42	Metcalfe, Ont., G.A.M. (2280); Ottawa, G.A.M. (1519)		28	(68, 69, 28) Eur.
					35	(68, 69, 15, 28) Eur.
				21		(67, 78) Eur.
					42	(67, 75, 25, 68, 69, 78, 15, 28, 74) Eur.; (82, 83) Asia
					56	(67, 69, 28) Eur.

TABLE I (*Concluded*)

Chromosome numbers of 38 Canadian weeds and references to previous counts

Species counted	Author's count		Source of material, collector, and author's collection number	Previous counts		References to previous counts
	n	2*n*		*n*	2*n*	
intermedia L. var. *canescens* Ruprecht		42	Lot 17, Conc. 7, Frontenac Co., Ont., G.A.M. (1519)	21		(78) Eur.
					42	(82, 83, 15, 78) Eur.
norvegica L.		70	Coaldale, Alta., D.R.L. (2154)		70	(24, 56) Eur.
recta L.		28	Ottawa, C.F. (1530); Midland, Ont., I.J.B. (2135); Okanagan Falls, B.C., H. Rhodes (2156); Monte Creek, B.C., G.A.M. (2207)		28	(75, 15) Eur.
					42	(82, 83) Asia; (15) Eur.
Scrophulariaceae						
Veronica persica Poir.		28	Chilliwack, B.C., D.F. (1511)	14		(34, 85) Eur.
					28	(5, 6, 7, 56) Eur.
Solanaceae						
Solanum dulcamara L.		24	Ottawa, G.A.M. (1441)	12		(97) Eur.
					24	(42, 17, 94) Eur.
Umbelliferae						
Carum carvi L.		20	Cardston, Alta., D.R.L. (2157)	10		(98, 29) Eur.
					20	(92, 22, 56) Eur.
				11		(62, 79) Eur.
Pastinaca sativa L.		22	Ottawa, G.A.M. (1521)	11		(73) Asia
					22	(62, 18, 92, 74) Eur.; (8) U.S.A.

Discussion

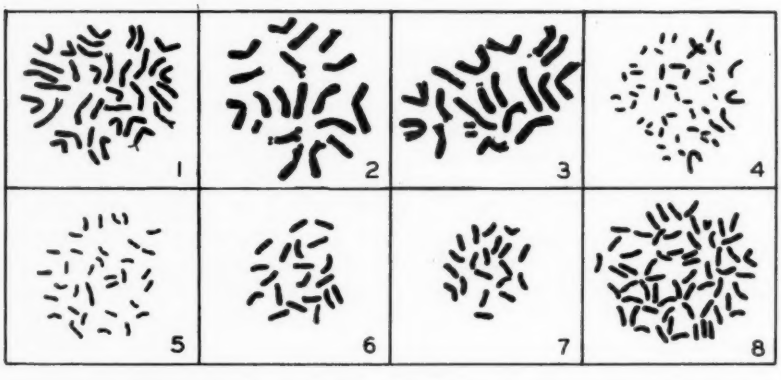
Thirty-eight of the taxa examined were previously counted by other workers; however, only six of these counts were determined on Canadian material. The somatic numbers of 20 for *Rumex transitorius* and *Rumex triangulivalvis*, and 60 for *Rumex verticillatus*, are the first determined on these species. From the author's counts and those of previous workers, it appears that 12 of the weeds examined have two or more different chromosome numbers. The 12 taxa (with the chromosome numbers of Canadian collections and, in parenthesis, all known chromosome numbers) are as follows: *Chrysanthemum leucanthemum* 18, 36 (18, 36, 54); *Senecio jacobaea* 40 (32, 40); *Erysimum hieraciifolium* 48 (32, 48); *Nepeta cataria* 34 (32, 34, 36); *Plantago major* 12 (12, 24); *Rumex acetosella* 42 (14, 28, 42, 56); *Rumex longifolius* 60 (60, 80); *Ranunculus acris* 14 (12, 14, 16, 28, 56); *Potentilla argentea* 28, 42 (14, 28, 35, 42, 56); *Potentilla recta* 28 (28, 42); *Carum carvi* 20 (20, 22). Chromosome numbers determined on Canadian material of the remaining 23 taxa are the same as the numbers reported by European, Asiatic, and American workers.

Chenopodium glaucum var. *salinum*, native to North America, and *C. glaucum* var. *glaucum*, native to Europe, both have a somatic chromosome number of 18. The chromosome number of var. *salinum* was previously reported by Å. Löve (53). This taxon is very common in the provinces of Manitoba, Saskatchewan, and Alberta and in eastern British Columbia. It

is very sparingly introduced at a few localities in the eastern provinces of Quebec and Ontario. The European variety, *glaucum*, is confined to the eastern provinces of Prince Edward Island, New Brunswick, Ontario, and Quebec.

The *Chrysanthemum leucanthemum* complex was discussed in some detail by Mulligan (64). The common oxeye daisy is diploid and appears to be the typical *Chrysanthemum leucanthemum* L. s. str., introduced from Europe. Tetraploid plants have also been introduced into North America from Eurasia but stands are relatively few and localized. The tetraploid is most common east of Quebec city to Gaspé, Quebec, and in Nova Scotia. The taxonomic status of the tetraploids is still in doubt. Plants of diploid *C. leucanthemum* L. s. str. have basal leaves generally coarsely and irregularly toothed, and the middle and upper leaves usually narrowly oblong or oblanceolate, conspicuously subpinnatifid at the base. North American tetraploid plants have basal leaves usually spatulate-obovate and closely and regularly crenate; the middle and upper leaves are usually oblong or oblanceolate, coarsely crenate or dentate above with larger spreading teeth at the base. Pollen grains of tetraploid plants have approximately twice the volume of pollen grains from diploid plants.

Iva xanthifolia, $2n = 36$ (Fig. 1) is a native of North America. Negodi (71) reported a chromosome count of $n = 14$, presumably on Italian material introduced from North America. The only other chromosome count in the genus *Iva* was reported by Heiser and Whitaker (31), *Iva axillaris*, $n =$ ca. 16–17. It is, therefore, with the information now available, impossible to determine the basic chromosome number of the genus *Iva* or to discover whether there are two chromosome numbers in *Iva xanthifolia*.



FIGS. 1–8. Somatic chromosome complements, ×2150. FIG. 1. *Iva xanthifolia*, $2n = 36$. FIG. 2. *Matricaria maritima* var. *agrestis*, $2n = 18+1B$. FIG. 3. *Matricaria maritima* var. *agrestis*, $2n = 18+2B$. FIG. 4. *Erysimum hieraciifolium*, $2n = 48$. FIG. 5. *Nepeta cataria*, $2n = 34$. FIG. 6. *Rumex transitorius*, $2n = 20$. FIG. 7. *R. triangulivalvis*, $2n = 20$. FIG. 8. *R. verticillatus*, $2n = 60$.

The presence in Canada of two chromosome races in *Matricaria maritima* var. *agrestis*, of Eurasia, was first suspected when specimens in the Department of Agriculture, Ottawa, herbarium were mapped. A co-worker, Dr C. Frankton, noticed that this plant had usually been collected either in the prairie provinces of Manitoba, Saskatchewan, and Alberta or in the maritime provinces of Newfoundland, Prince Edward Island, Nova Scotia, New Brunswick, and also in southeastern Quebec. As this was an unusual distribution for a weedy species in Canada, he suspected that two different populations existed. Further investigation revealed that pollen grains of prairie material had approximately twice the volume of pollen grains from maritime material and that prairie plants had 36 somatic chromosomes while maritime plants had only 18. All the material studied was grown in nursery plots and many herbarium specimens were examined but no morphological characters were found that distinguished the diploids from the tetraploids. The diploid race was apparently introduced in ballast along the Atlantic seaboard and is now weedy in wasteland, farmyards, and along roadsides and railroad tracks. It is not a weed in agricultural lands. The tetraploid race probably escaped from gardens in the prairie provinces and has spread not only into wasteland and along roadsides but also, as a weed of some importance, into grainfields and pastures, particularly in Saskatchewan.

Accessory chromosomes were seen in the root tips of all the Prince Edward Island diploids examined but no accessory chromosomes were present in somatic plates of the diploids from Nova Scotia, New Brunswick, and Quebec or in any of the tetraploids. All five plants examined from Charlottetown, P.E.I., had one accessory chromosome (see Fig. 2) while four of the five plants examined from Kinkora, P.E.I., had one accessory and the other plant two accessory chromosomes (see Fig. 3). Therefore, all or a large portion of the material of *M. maritima* var. *agrestis* in Prince Edward Island has at least one accessory chromosome. The accessory chromosomes could have arisen in the diploids after they were introduced into Prince Edward Island. Diploids with accessory chromosomes could also have been introduced into Prince Edward Island and either there was selection for plants without accessories when the species spread into Nova Scotia, New Brunswick, and Quebec, or diploid plants without accessory chromosomes were introduced into the latter three provinces from a source different from that of the plants of Prince Edward Island. It seems most likely that *M. maritima* var. *agrestis* had accessory chromosomes when introduced into Prince Edward Island from a European source and plants of Nova Scotia, New Brunswick, and Quebec with the normal diploid complement represent a different introduction. As the maritime provinces are very alike in climate and habitats and there is no evidence of introgression or crossbreeding with related species in Prince Edward Island, it is very unlikely that diploid *M. maritima* var. *agrestis* has either undergone selection for plants without accessory chromosomes since its arrival in Canada or given rise to plants with accessory chromosomes after the introduction of normal diploids.

Erysimum hieraciifolium, $2n = 48$ (Fig. 4), an introduced weed of Eurasiatic origin, has become well established in some parts of eastern Ontario. The somatic counts of 32 by Jaretzky (39) and Löve and Löve (56) indicate that there are at least two chromosome races, tetraploid and hexaploid, in this species.

Plants of *Plantago major*, probably introduced from Europe, have 12 chromosomes in somatic cells. This is the diploid chromosome number which previous workers have reported for American and European material. Three tetraploid counts reported by Ikeno (37), Ishikawa (38), and Takahashi (91) were all on Japanese material identified as *Plantago major* var. *asiatica*. If tetraploid plants exist in Canada, they may be expected to occur along the Pacific coast.

Nepeta cataria, $2n = 34$ (Fig. 5) has been introduced from Europe. Previous counts on this species by Bushnell (14) and Suguira (88) were $n = 16$ and $n = 18$, respectively. Since the basic number of all other species of *Nepeta* is $x = 18$, it appears that the haploid number in some plants of *Nepeta cataria* has been reduced from 18 to 17 and 16. Somatic metaphase plates prepared from the plants collected in P.E.I., N.S., Ont., and B.C. had one pair of chromosomes always approximately twice the length of the other 16 pairs, and in the three drawings of chromosomes at meiotic diakinesis in Bushnell (14), two chromosome pairs appear larger than the other 14 pairs. This suggests that the reduction in chromosome number in *Nepeta cataria* might have arisen by the fusion of chromosomes.

Rumex acetosella from two locations in Ontario, one in Saskatchewan, and three in British Columbia had 42 somatic chromosomes. Löve (50, 52) has placed the chromosome races of the *R. acetosella* complex in the following species: *R. angiocarpus* Murbeck ($2n = 14$), *R. tenuifolius* (Wallr.) Löve ($2n = 28$), *R. acetosella* L. s. str. ($2n = 42$), and *R. graminifolius* Lambert ($2n = 56$). However, the only morphological character separating *A. angiocarpus* from *A. acetosella* L. s. str. is the fusion of the valve to the seed in the former species and non-fusion of valve to seed in the latter species. In some Canadian material the valve is fused to the seed, presumably placing these plants in *A. angiocarpus*, which Löve reports has 14 somatic chromosomes. However, when somatic material of such a specimen was examined (see Penticton, B.C. (2243) in Table I), 42 somatic chromosomes were found, the number of *R. acetosella* L. s. str. As there is also no way of identifying male plants, the author considers the material counted simply a hexaploid race of the *R. acetosella* complex, introduced from Europe.

Rumex transitorius, $2n = 20$ (Fig. 6), *R. triangulivalvis*, $2n = 20$ (Fig. 7), and *R. verticillatus*, $2n = 60$ (Fig. 8) belong to the subgenus *Rumex*, section *Axillares*, Rech. f. and all are native to North America. Chromosome counts on *R. salicifolius*, section *Axillares*, by Jaretzky (40) and Kihara (44) were both $n = 10$. These chromosome counts indicate that this section has a base number of $x = 10$, the same as that of section *Rumex* (= *Simplices* Rech. f.) of the subgenus *Rumex*. A close relationship between taxa in

sections *Axillares* and *Rumex*, the former section almost exclusively native to North America and the latter section mostly native to Eurasia, is indicated. This relationship is further substantiated by an apparent hybrid between *R. triangulivalvis*, $2n = 20$ (sect. *Axillares*) and *R. fennicus*, $2n = 40$ (sect. *Rumex*) collected near Kindersley, Saskatchewan, by Dr. C. Frankton. The chromosome number of $2n = 30$ determined from root tips of this plant substantiates its suspected hybrid nature.

Potentilla argentea, introduced from Europe, from three locations, two in Ontario and one in Quebec, were examined. Plants from both Ontario locations had 42 somatic chromosomes while the Quebec plants had 28 somatic chromosomes. From Table I, it can be seen that chromosome races with 14, 28, 35, 42, and 56 somatic chromosomes have been found in Europe. Müntzing and Müntzing (68, 69) found no morphological characters that distinguished the chromosome races. They found many morphological strains at each polyploid level and noted that the strains were remarkably uniform, a condition that they attributed to apomixis. As material was examined from only three locations, it is not known how many chromosome races are present in Canada or even if the races reported here are the common ones.

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STUDIES OF WESTERN TREE RUSTS

IV. UREDINOPSIS HASHIOKAI AND U. PTERIDIS CAUSING PERENNIAL NEEDLE RUST OF FIR[1]

W. G. ZILLER[2]

Abstract

Observations in the field and the results of inoculation experiments show that perennial needle rust of fir (*Abies* spp.), known as *Peridermium pseudo-balsameum* (Diet. & Holw.) Arth. & Kern, is caused by *Uredinopsis hashiokai* Hirats. f. and *U. pteridis* Diet. & Holw. (*U. macrosperma* Magn.), which complete their life cycles on bracken fern (*Pteridium aquilinum* (L.) Kühn var. *lanuginosum* (Bong.) Fern.). The pycnial and aecial states of *Uredinopsis hashiokai* are described, and for the first time the life histories of *U. hashiokai* and *U. pteridis* are presented. The two species are indistinguishable from each other in life history, host relationship, and morphology of most of the spore states; they differ from each other in their geographical distribution and in the morphology of their urediniospores. It remains unknown which of the two species of *Uredinopsis* represents the perfect state of *Peridermium pseudo-balsameum*. *Uredinopsis aspera* Faull proved to be a later synonym of *U. hashiokai*. *U. hashiokai* is noteworthy because of its similarity to *U. pteridis*, and both species are unusual in development of their spore states on fir, particularly in the long periods required for maturation of the aecia, which are produced from localized, perennial mycelium 4 to 11 months after infection in *Abies lasiocarpa* (Hook.) Nutt. and *A. grandis* (Dougl.) Lindl. respectively.

Introduction

The identification of the white-spored rust fungi (species of *Milesia* and *Uredinopsis*) from their pycnial and aecial spore states on fir (*Abies* spp.) has always been considered difficult. It has been attempted by Kamei (9, pp. 91 and 148), who has presented keys to six species of *Milesia* and nine species of *Uredinopsis* occurring on fir in Hokkaido, Japan. The characteristics in the keys were derived entirely from the position and morphology of the pycnia and aecia, particularly the markings of the peridial cells. Hunter (7, 8) has given detailed descriptions and photomicrographs of the pycnia of 15 species of *Milesia* and *Uredinopsis*, four of which are known to occur in western North America. She has demonstrated convincingly the relatively high value of pycnia as an aid to the taxonomic classification of the fir rusts. In view of the above studies and our ever-increasing knowledge of the life histories of western rusts, it is to be expected that sufficient taxonomic data will become available to enable us to recognize the white-spored peridermia on fir and to refer them to the fern rusts which represent their telial states.

Until recently, the only experimental evidence indicating that the fir needle rust *Peridermium pseudo-balsameum* is the aecial state of the fern rust *Uredinopsis pteridis* consisted of one experiment, in which Weir and Hubert (12) sowed aeciospores of *Peridermium pseudo-balsameum* on fronds of bracken

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fern and obtained uredinia of *Uredinopsis pteridis*. In 1955, Solheim and Cummins (11, p. 157) repeated Weir and Hubert's experiment, obtaining uredinial infections of *U. pteridis* by inoculating bracken fern with aeciospores from grand fir (*Abies grandis* (Dougl.) Lindl.) in Montana. When the writer tried to repeat Weir and Hubert's experiment, however, the uredinial infections he obtained on the fern (in expt. A, Table I) proved to be not *Uredinopsis pteridis* as expected but *U. aspera*, a rust of bracken fern hitherto unconnected (4)! It will be shown (in section Taxonomy) that *U. aspera* is a later synonym of *U. hashiokai* (6).

The unexpected result from the inoculation of bracken fern prompted the writer to initiate further research in the taxonomy and life histories of the bracken fern rusts.

Methods

The methods and material used for inoculation were essentially the same as described in a previous publication (13). Aeciospores from the peridermia on fir were brushed on the moistened fern fronds, after which the fern was kept in a moist chamber, provided by a plastic bag that was removed after 2 to 3 days. The material used for telial inoculum was overwintered near ground level in the field and placed in moist chambers for 2 to 5 days, or until germination of the telia began. Then, after the telial material was placed on galvanized wire screen which was suspended over the young needles of the fir, it was thoroughly moistened and enclosed with the fir in a plastic bag. The bag and the screen with the inoculum were removed in about three days.

The test plants and their controls were left either in the greenhouse after inoculation or in the field ("g" and "f" in Table I) at a place where natural infection with rust spores was not likely to occur. The controls were prepared ahead of the test plants to avoid chance infection of control plants with inoculum. The greenhouse was protected from insects by screen windows, well ventilated, and not heated. The greenhouse plants were watered regularly from below, and care was taken to protect them from high temperature and direct sunlight, particularly during the inoculation period. Pycnia on the needles of part of the branches of fir in the greenhouse were spermatized; the pycnial drop on each pycnium was touched in turn with the tip of a toothpick, with the object of transferring pycniospores of the opposite mating type to each pycnium. This was done to test the necessity of such an artificial transfer for subsequent formation of aecia under greenhouse conditions.

Urediniospores provided the only reliable criteria by which bracken fern rusts can be identified. With the possibility in mind that a specimen might contain more than one species, spores from only one uredinium were included in each spore mount. Approximately ten spore mounts from each specimen representing an experiment were examined.

The photomicrographs in Plates I–III were made of culture material from expts. C (Fig. 2), D (Figs. 1, 3, and 4), E (Figs. 16–18), F (Figs. 5 and 6), I (Figs. 7 and 8), J (Figs. 9–12), L (Figs. 22–24), M (Figs. 19–21), and of type

material of *Uredinopsis hashiokai* (Figs. 13–15). For magnifications of ×1000, with the exception of Figs. 2 and 8, a Reichert photomicrographic camera "Kam VBX" mounted on a "Zetopan" microscope with green light filter and phase contrast illumination was used. The specimens were mounted in lactophenol, except the specimens for Figs. 2, 8, 22, 23, and 24. The pycniospores shown in Figs. 2 and 8 were obtained from fresh pycnia and mounted in their pycnial exudate, while the urediniospores shown in Figs. 22–24 were mounted in 5% KOH and heated to the boiling point in order to show the spore markings and position of the four germ pores more clearly. Following this treatment the spore walls appeared abnormally thickened (Figs. 23 and 24).

TABLE I

Inoculation experiments with *Uredinopsis hashiokai* and *U. pteridis*

Expt.	Inoculum	Host inoculated, location	Date of inoculation	No. of days to appearance of: O	I	II
		Inoculations with *Uredinopsis hashiokai*				
A	I from *Abies grandis* field collection	*Pteridium*, f	May 12, 1952			20
B	I from *Abies grandis* field collection	*Pteridium*, f	June 18, 1954			21
C	IV from *Pteridium*, from expt. B	*Abies grandis*, f	May 30, 1955	35	358	
		A. grandis, g	May 30, 1955	21	263	
		A. amabilis, g	May 30, 1955	—	—	
D	I from *Abies grandis* field collection	*Pteridium*, f	June 11, 1955			23
E	I from *Abies grandis* field collection	*Pteridium*, f	June 11, 1955			23
F	IV from *Pteridium*, from expt. D	*Abies grandis*, g	May 7, 1956	25	332	
		A. lasiocarpa, g	May 7, 1956	25	156	
G	I from *Abies grandis*, from expt. C	*Pteridium*, f	May 23, 1956			18
		Inoculations with *Uredinopsis pteridis*				
H	I from *Abies grandis* field collection	*Pteridium*, f	May 24, 1955			21
I	IV from *Pteridium* field collection	*Abies grandis*, g	May 30, 1955	21	268	
		A. amabilis, g	May 30, 1955	?43	168	
J	IV from *Pteridium* field collection	*Abies grandis*, g	May 7, 1956	25	—	
		A. grandis, f	May 13, 1956	35	345	
		A. lasiocarpa, g	May 7, 1956	25	106	
K	IV from *Pteridium*, from expt. H	*Abies grandis*, g	May 7, 1956	25	332	
		A. lasiocarpa, g	May 7, 1956	—	—	
L	I from *Abies grandis*, from expt. I	*Pteridium*, f	May 23, 1956			18
M	I from *Abies lasiocarpa*, from expt. J	*Pteridium*, f	Aug. 21, 1956			20

Note: The Roman numerals O–IV denote the *mature* pycnial, aecial, uredinial, telial, and basidial spore states, respectively.
The "location", third column, denotes the place of inoculation: f, field; g, greenhouse.

Inoculations

Obviously more experimental evidence, in the form of data and specimens obtained from inoculations, was needed to test the apparent relationship between *Peridermium pseudo-balsameum* and the rusts of bracken fern. Consequently, 13 inoculation experiments were carried out in Victoria, B. C., and vicinity over a period of 5 years. Comments on the experiments, summarized in Table I, follow below.

Experiment A

The inoculum was collected in the vicinity of Victoria. One of the fern fronds was inoculated with aeciospores from fir needles of the previous year's (1951) growth, the other with aeciospores from 2-year-old (1950) needles. The two fronds were incubated in separate plastic bags. From the infections obtained on both fronds, uredinia of *Uredinopsis hashiokai* (*U. aspera*) developed in abundance. One month after inoculation, *U. hashiokai* was collected on bracken fern at the same place where the inoculum was collected on grand fir.

Experiments B, C, and G

The inoculum for expt. B was also collected near Victoria, but it consisted of aeciospores on the needles of only the previous year's growth; the inoculum for expt. C consisted of basidiospores from telia in the fern obtained by inoculation in expt. B; and the inoculum for expt. G consisted of aeciospores on needles of the previous year's (1955) growth of grand fir obtained by inoculation in expt. C (Table I). In expts. B and C the rust fungus completed its life cycle on both hosts, and in expt. G it was induced to repeat its life cycle up to the telial state in the fern. The whole course of development, from the aecial to the uredinial, telial, and basidial states on fern to the pycnial and aecial states on fir and back to the uredinial and telial states on fern, was initiated from the aecial inoculum for expt. B. All phases of development took place in the field under normal field conditions. Two infected grand fir were kept alive for 2 years, 1 in the field and 1 in the greenhouse, and the following observations were made on them. The appearance of pycnia was accompanied by slight yellowing of the infected needles and formation of pycnial drops. Exudation of pycnial fluid continued almost throughout the experiment even on the needles which had produced mature aecia. The drops were clearly visible each morning in the greenhouse, but they were washed off by dew and rain in the field. In the greenhouse aecia matured only on needles which had been spermatized; in the field, where no artificial spermatization was carried out, aecia matured on most infected needles. The periods required for maturation of pycnia and aecia (Table I) were considerably longer in the field than in the greenhouse, but the rupture of the mature aecial columns occurred sooner in the field, presumably because of rain and dew. Some aecia in the greenhouse reached 8 mm in length before they finally ruptured, 18 months after inoculation! Dehiscence of the peridium occurred by formation of lateral slits.

In the greenhouse, most of the heavily infected needles died during the summer after inoculation, while only a few needles died during the same period on the test plant in the field. However, many infected needles died in the field during the following severe winter. No new aecia appeared on any needles in the greenhouse and in the field during the second season, that is, after May, 1956. This was determined by close examination of the aecia-bearing needles, each of which was marked with paint in May, 1956. The aecia which had appeared in the spring of 1956 resumed their growth in the spring of 1957 after a period of winter dormancy. The rust mycelium appears to remain perennial in the living needle without spreading to adjacent needles.

The rust on fern in expts. C and G was identified as *Uredinopsis hashiokai*; but the specimens obtained by inoculation in expt. B contained an admixture of *U. pteridis*, which was absent from the specimens obtained in expt. G. No infection of amabilis fir (*Abies amabilis* (Dougl.) Forb.) was obtained. Differences of results between greenhouse and field inoculations are ascribed mainly to differences in environment.

Experiments D and F

These two experiments are repetitions of expts. B and C. Their results essentially confirm the observations on the earlier experiments outlined above. The inoculum for expt. D was collected near Victoria, on fir that was surrounded by bracken fern rusted with *Uredinopsis hashiokai*. The inoculum for expt. F was obtained by inoculation of fern in expt. D. No inoculations of fir were made in the field.

Alpine fir (*Abies lasiocarpa* (Hook.) Nutt.) was artificially infected with a bracken fern rust for the first time, at the same date as the grand fir (expt. F). The reaction of the alpine fir differed significantly from that of grand fir; 5 months after inoculation, aecia already had begun to mature on many of the needles which had been spermatized. Over 30% of the infected needles (bearing pycnia) died a month after inoculation, and very few infected needles were alive after 1 year. As a rule, pycnia and aecia were borne amphigenously on those needles of alpine fir which had stomata on both surfaces of the needle.

The rust, as determined from 15 mounts of urediniospores, was identified as *Uredinopsis hashiokai* without an apparent admixture of *U. pteridis*.

Experiment E

The experiment represents the fifth replication of expt. A and confirms observations outlined above. No admixture of *U. pteridis* was detected in the uredinia of the infected fern.

To facilitate comparison between the two species, most of the following experiments (with *U. pteridis*) were carried out under the same conditions and at the same time as experiments with *U. hashiokai* outlined above. No rust other than *U. pteridis* could be detected in the material for expts. H to M.

Experiments H and K

The inoculum for expt. H was collected at Genoa Bay, about 40 miles north of Victoria, at a location where exceptionally good specimens of *U. pteridis* were collected on bracken fern later, in July, 1955. Expts. H and K did not reveal any difference between the life histories of *U. pteridis* and *U. hashiokai*. The striking similarity between the two species was borne out further by the following experiments.

Experiments I and L

The inoculum for expt. I was collected at the same time and place as the inoculum for expt. H, above: the bracken fern in the field containing uredinia and telia of *U. pteridis* surrounded the fir seedling, which was obviously infected with the rust from the fern. The methods and results in expts. I and C concerning the inoculation of grand fir in the greenhouse seem to be identical. The rust on grand fir was observed for a period of 2 years in expt. C as well as in expt. I. The life cycle was completed (expts. L and G) by infecting fern with aeciospores from grand fir obtained by inoculation (in expts. I and C). Infection of amabilis fir, although sparse, was obtained for the first time in expt. I.

Experiments J and M

The two experiments were carried out to compare the form of *U. pteridis* occurring in the interior regions of British Columbia with the form occurring on the coast. The two forms seem to differ significantly in the morphology of the urediniospores (Figs. 19–24). Bracken fern rusted with "long-spored" *U. pteridis* (Figs. 19–21) was collected near Aleza Lake, 400 miles north of Victoria, late in fall and used as inoculum (expt. J) in the following spring, after being overwintered near Victoria in the usual manner. Of the two grand fir seedlings inoculated in expt. J, the seedling in the greenhouse became only sparsely infected, developing pycnia but no aecia, while the seedling in the field developed both pycnia and aecia, in exactly the same manner as the grand fir inoculated in the field in expt. C.

The alpine fir seedlings used as test plants were obtained from Aleza Lake. The infection of alpine fir in expt. J was exceptionally abundant and remarkable for the relatively short period required for maturation of the aecia. There were indications that alpine fir is more tolerant to "long-spored" *U. pteridis* than to *U. hashiokai* (expt. F) under apparently similar conditions in the greenhouse. The urediniospores of the fern rust obtained in expt. M and urediniospores seen in the material used as inoculum for expt. J were of the same type (Figs. 19–21). Experiments J and M show that, under certain conditions at least, *U. pteridis* can complete its life cycle in 1 year. Evidence that the rust completes its life cycle in 1 year under natural conditions is lacking.

Controls for all experiments remained free of rust. All 59 specimens used as inocula and obtained from inoculation are filed in the Mycological Herbarium of the Forest Biology Laboratory, Victoria, B. C. (DAVFP).

Taxonomy

From the results of Weir and Hubert's (12), Solheim and Cummins' (11), and the writer's inoculations it would appear that two species of *Uredinopsis*, *U. hashiokai* and *U. pteridis*, occur on bracken fern in British Columbia, both of which cause perennial needle rust on the firs. In the light of this experimental evidence it became desirable to review the taxonomy of these two species and their relation to *U. aspera* Faull, a bracken fern rust closely resembling *U. hashiokai*, and *Peridermium pseudo-balsameum*, assumed to be the aecial state of *U. pteridis*. For that purpose the following specimens were procured:

Uredinopsis pteridis on *Abies grandis* at Priest River, Ida., June 17, 1916, J. R. Weir. Material used for infecting *Pteridium* (12). BPI.[3]

U. pteridis on *Pteridium aquilinum* var. *lanuginosum* at Missoula, Mont., July 25, 1916, J. R. Weir, No. 884. Obtained by inoculation with aeciospores from Priest River, Ida. (12). BPI.

U. aspera on *Pteridium aquilinum* var. *lanuginosum* at Pasadena, Calif., Jan. 25, 1893, A. J. McClatchie. Type, at Herb. Faull (4).

U. hashiokai on *Pteridium aquilinum* var. at Tainan, Formosa, Nov. 6, 1932, Y. Hashioka. Type, at Herb. Hiratsuka (6).

U. hashiokai on *Pteridium aquilinum* var. at Taihoku, Formosa, July 29, 1934, Y. Hashioka. At Herb. Hiratsuka (6).

The first two collections listed represent Weir and Hubert's cultural material. Faull compared these specimens with the types of *Peridermium pseudo-balsameum*, *Uredinopsis pteridis*, and *Uredo macrospermum* Cooke and concluded that "unquestionably the rust of Weir and Hubert's experiments is *Uredinopsis macrosperma* (Cooke) Magnus (*Uredinopsis Pteridis* D. & H.) and its haploid phase is *Peridermium pseudo-balsameum* (D. & H.) Arth. & Kern" (4, p. 92). The collection on bracken fern is sparse, which is to be expected from the "medium infection" (12, p. 330) obtained by Weir and Hubert, but both specimens, the aecial inoculum used and the uredinial infection obtained, are well preserved. Urediniospores of only *Uredinopsis pteridis* were found in the culture material on fern representing Weir and Hubert's and the writer's experiments (expts. H–M). The pycnia and aecia in the material on grand fir agreed morphologically with *Peridermium pseudo-balsameum* in Faull's monograph (4, p. 86), with the exception that the pycnia (spermogonia) occur on needles of the first year, also, and that the peridium has been observed to rupture laterally. Experiments H to M thus confirm Weir and Hubert's discovery of the aecial state of *Uredinopsis pteridis* on grand fir.

A brief review of the rusts of the world on *Pteridium* shows that two groups of species of *Uredinopsis* occur on several varieties of *Pteridium aquilinum*. The two groups are recognized mainly by the presence (for example *Uredinopsis pteridis*) or absence of "two opposed vertical rows of cogs" (4, p. 32)

[3]Lanjouw and Stafleu (10) are followed for abbreviations of names of herbaria.

on the urediniospores. *U. hashiokai* and *U. aspera*, sole representatives of the latter group, also lack amphispores. They differ from each other morphologically in the length and width of their urediniospores (4, Figs. 17 and 18). Slight differences in thickness of the urediniospore wall have also been noted. *U. hashiokai*, moreover, is known only from Formosa; *U. aspera* from the west coast of North America and from Hawaii.

TABLE II

Measurements of urediniospores of *Uredinopsis aspera* and *U. hashiokai*

Source	Collected		Spore size, μ		Remarks
	Place	Month	Range	Average	
Publications:					
Faull (4)	Cal., B. C. Hawaii	—	14–30×24–46	20×32	*U. aspera*
Faull (4)	Formosa	Nov.	16–20×30–58	17×43	*U. hashiokai*
Hirat. (6)	Formosa	—	14–18×33–60	—	*U. hashiokai*
Original:					
Faull 11210	Cal.	Jan.	16–28×20–50	19×34	p, *U, aspera*, type
Faull 11210	Cal.	Jan.	15–28×20–43	20×33	p, *U. aspera*, type
B.F.C. 2988	Cal.	Oct.	18–26×26–41	20×34	t, exsiccati
B.F.C. 2988	Cal.	Oct.	17–22×28–46	20×31	p, exsiccati
B.F.C. 4087	B. C.	Sept.	16–21×26–42	19×34	t, exsiccati
B.F.C. 4787	Cal.	Aug.	18–24×26–38	21×32	t, exsiccati
B.F.C. 4787	Cal.	Aug.	19–29×25–34	22×31	p, exsiccati
B.N.A.U. 881	B. C.	Sept.	16–22×29–50	18×37	t, exsiccati
B.N.A.U. 1682	Cal.	Aug.	15–25×25–39	22×30	t, exsiccati
B.N.A.U. 1682	Cal.	Aug.	16–25×26–45	19×35	p, exsiccati
TRTC	Ore.	July	14–26×26–55	19×36	p, Jackson, 1082
TRTC	Ore.	Aug.	14–24×26–52	19×38	p, Jackson, 1058
TRTC	Ore.	Sept.	16–24×25–52	20×39	t, Jackson, 1142
TRTC	Ore.	Sept.	17–24×24–38	20×31	p, Jackson, 1142
DAVFP 3213	B. C.	June	16–25×25–47	20×34	t
DAVFP 6381	B. C.	Sept.	16–25×26–42	21×34	t
DAVFP 7159	B. C.	Sept.	15–20×25–49	18×36	t
DAVFP 7844	B. C.	June	16–23×30–50	18×42	t
DAVFP 7844	B. C.	June	14–22×28–44	18×37	t
DAVFP 7844	B. C.	June	14–20×31–62	18×43	t
DAVFP 7858	B. C.	June	15–20×29–46	18×37	t
DAVFP 7965	B. C.	June	14–21×28–46	18×37	t, expt. A
DAVFP 7974	B. C.	June	14–20×32–49	17×40	t, expt. A
DAVFP 8015	B. C.	Sept.	14–20×32–56	17×41	t, expt. A
DAVFP 8033	B. C.	Sept.	13–23×28–46	19×39	t
DAVFP 8033	B. C.	Sept.	13–24×20–33	20×26	Old pustule
DAVFP 8061	B. C.	Sept.	14–21×26–47	19×36	t
DAVFP 8250	B. C.	Oct.	17–25×26–40	20×31	t
DAVFP 9233	B. C.	July	13–24×27–50	17×36	t, expt. B
DAVFP 9234	B. C.	July	13–20×34–66	16×50	t, expt. B
DAVFP 9236	B. C.	July	18–24×33–52	21×41	t, expt. B
DAVFP 9253	B. C.	July	14–20×25–55	17×41	t
DAVFP 9293	B. C.	July	14–22×30–57	18×39	t, expt. B
Hirat. 116	Formosa	Nov.	12–19×24–40	17×32	p, *U. hash.*, type
Faull 13012	Formosa	Nov.	13–21×30–52	17×39	t, *U. hash.*, type
Hirat.	Formosa	July	12–19×22–46	16×33	p, see ref. 6
Total, original measurements			12–29×20–66	19×36	Approx. 1000 spores measured

Note: p, spores from the pustule of one uredinium;
t, spores from the tendril of one uredinium.
Lanjouw and Stafleu (10) are followed for abbreviations of names of herbaria.
B.F.C., E. Bartholomew, Fungi Columbiani.
B.N.A.U., E. Bartholomew, North American Uredinales.

The remaining three collections listed above represent authentic specimens of *U. aspera* and *U. hashiokai* on varieties of *Pteridium aquilinum*. They were compared with the 15 fern rust specimens obtained by inoculation in expts. A to G, and 31 field collections of "*U. ?hashiokai*" from California, Oregon, and British Columbia to determine the taxonomic relationship between *U. aspera* and *U. hashiokai*.

Table II has been prepared to facilitate the comparison of urediniospores from specimens of various origins. Except for the dimensions derived from publications, each line represents measurements of 20 to 40 spores from a single uredinium. Extruded spores from spore tendrils were examined in preference to spores still contained within pustules. Wall thickness of the base and apex of the spores, being of no apparent diagnostic value, was disregarded. The average thickness of the side walls, although not listed, was determined for each spore mount.

The extreme variability in the lengths of urediniospores contained in one pustule did apparently not become evident until recently, when sufficient representative material became available for study. The exsiccati cited by Faull (4) as well as the type of *U. aspera* (Table II) contain a relatively large percentage of short, broadly obovoid spores, but long and clavate spores are always present. The highest percentage of narrowly clavate and occasionally angled spores were noted in specimens collected in early summer from vigorous, young host plants up to six feet high growing in humid and shaded locations. A wide range in urediniospore dimensions is not surprising; it is the rule rather than the exception for species of the genus *Uredinopsis*.

In addition, several other features by which *U. aspera* might be distinguished from *U. hashiokai* were investigated, including dimensions and spacing of the projections from the urediniospore walls, uredinial peridia, telia, and teliospores, and observations made during expts. A to G.

From this comparative analysis the conclusion was drawn that *U. aspera* can no longer be considered as a species distinct from *U. hashiokai*. Since *U. hashiokai* has nomenclatural priority over *U. aspera*, the former name is retained. An amended diagnosis, based on the collections listed in Table II and the specimens obtained by inoculation in expts. A–G, is appended:

URedinopsis hashiokai Hiratsuka f., Mem. Tottori Agr. Coll. 4, 82 (1936). O, I, II, and III.

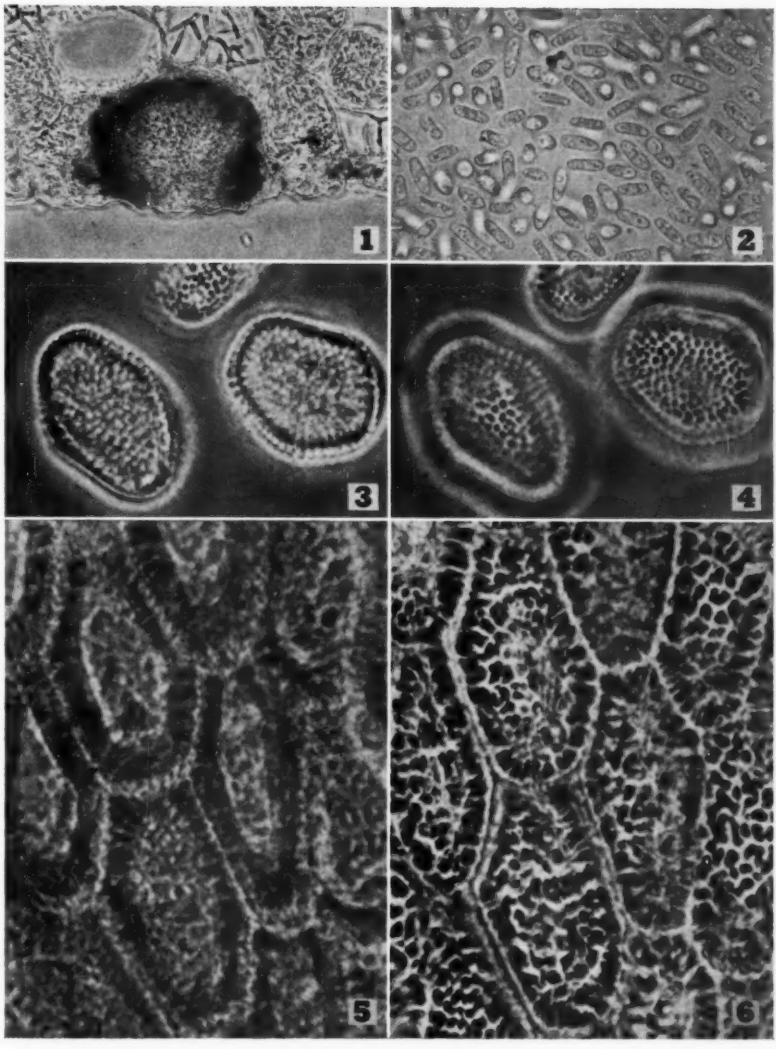
Uredinopsis aspera Faull, Arnold Arboretum Contrib. 11, 79 (1938). II and III.

Pycnia (Fig. 1) on needles 1 month to 4 years old, hypophyllous (on *Abies grandis*) or amphigenous (on *A. lasiocarpa*), numerous, scattered among the aecia, amber-green, subcuticular and covered by the combined cuticle and outer epidermal wall; immersed, inverted hemispherical but vertically elongated, plane at leaf surface, apical opening a short slit, 90–160 μ wide × 100–200 μ long × 70–135 μ high. Pycniospores (Fig. 2) straight, cylindrical, hyaline, 1.5–2.5 × 4–6.5 (8.0) μ.

Aecia on needles 10 months to 4 years old and hypophyllous (on *A. grandis*), or 5 months old and older and amphigenous (on *A. lasiocarpa*), biseriate, white, cylindrical, fragile, 0.2–0.5 mm in diameter, up to 2.5 mm (8 mm in greenhouse) high, arising in spongy parenchyma of mesophyll at the base of the palisade layer. Peridium hyaline, dehiscent laterally and becoming lacerate. Peridial cells in a single layer, overlapping, 15–30 μ wide × 30–70 μ long excluding basal and apical cells; seen in tangential section of the aecium (Fig. 5) rhomboid to polygonal, vertically elongate, side walls including warts 4–8 μ thick but considerably thicker (up to 20 μ) at the apex, outer wall smooth, inner wall very coarsely and closely verrucose (Fig. 6), appearing reticulate, the warts irregularly polygonal to elongate, 0.5–3.0 μ wide × 0.5–4.0 μ long × 1–5 μ spacing from center to center; seen in radial section of the aecium, outer wall 1.0–1.5 μ thick and inner wall 7–12 μ thick including warts, warts 4–10 μ high; apical cells spore-like, often detached, thick-walled with eccentric lumen. Aeciospores (Figs. 3 and 4) subspherical to obovoid and oblong, more often ellipsoid and irregularly angled, 15–22 × 22–31 (33) μ. Aeciospore wall with a smooth spot which is not caused by deciduous warts, verrucose, evenly 0.5–0.75 μ thick excluding warts; warts seen in spore surface view more or less isodiametric, rarely elongate, polygonal to stellate, not confluent, not crowded, evenly distributed excluding the smooth spot, (0.5) 0.75–1.5 (2.0) μ in diameter × 1–2 μ (average 1.4 μ) spacing from center to center; warts seen in median section of spore rectangular (Fig. 3), 0.0–1.5 (2.0) μ (average of 1.2 μ) high, gradually decreasing in height toward the smooth spot.

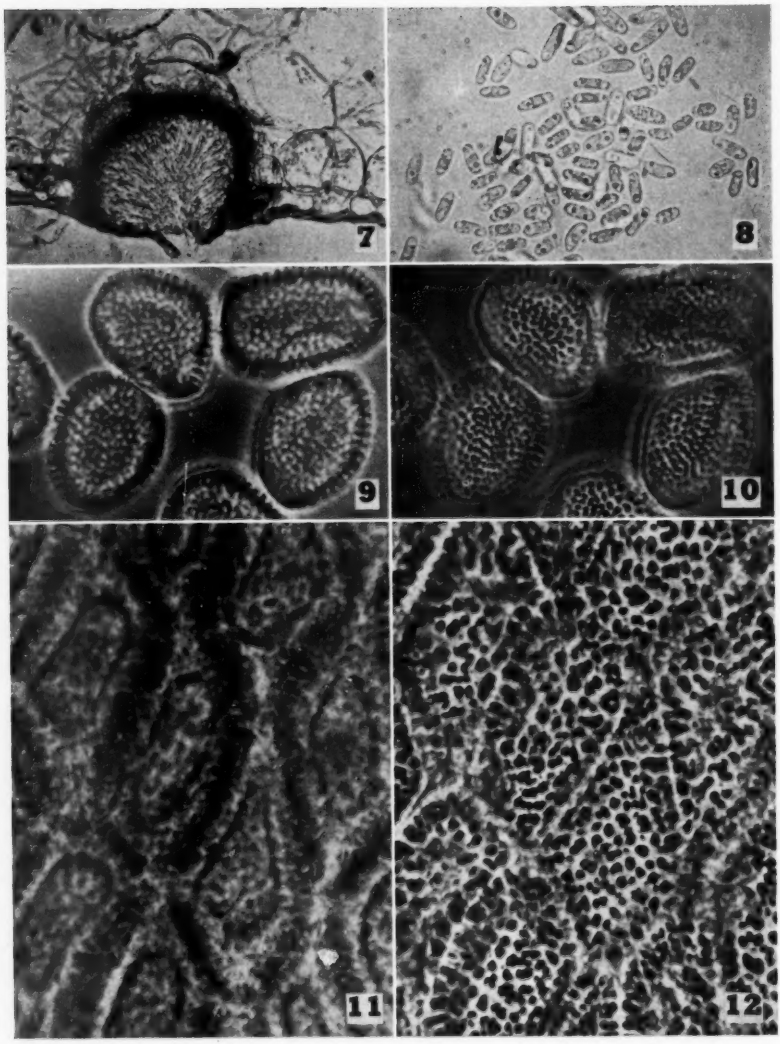
Uredinia hypophyllous, subepidermal, scattered or loosely grouped on yellow to brown discolored intercoastal areas, round to linear, 0.2–2.5 mm in largest axis, covered by yellowish-brown epidermis, dehiscent from a central or lateral rupture of the epidermis, convex, hyaline. Peridial cells in a single layer, isodiametrically to irregularly polygonal in surface view of peridium, 8–16 μ wide × 10–20 μ long, walls about 2 μ thick. Urediniospores (Figs. 13–18) hyaline, white in mass when fresh, extended in bulky masses or in tendrils, varying from subglobose and broadly obovoid to pyriform, ellipsoid, and clavate, the narrowest spores occasionally angled apically, never mucronate, most commonly pyriform and truncate at base, (12) 14–24 (29) × (20) 24–48 (66) μ (about 2000 spores measured). Spore walls sparsely but prominently echinulate. Side walls of urediniospores evenly 0.5–2.0 μ (average 1.0 μ) thick excluding spines, the apical and basal walls occasionally thicker, particularly at the apices of the angled spores; spines conical, rounded at the apex, irregularly spaced, 0.5–1.5 μ basal diameter × 0.5–1.0 μ high × 1–5 μ (average 2 μ) spacing from center to center.

Telia diffuse, mostly hypophyllous. Teliospores subepidermal, intercellular, scattered or loosely aggregate in a single layer, subglobose to globose, 1- to 4-celled, usually cruciate, 15–26 μ in diameter. Teliospore wall hyaline, smooth, about 1 μ thick.



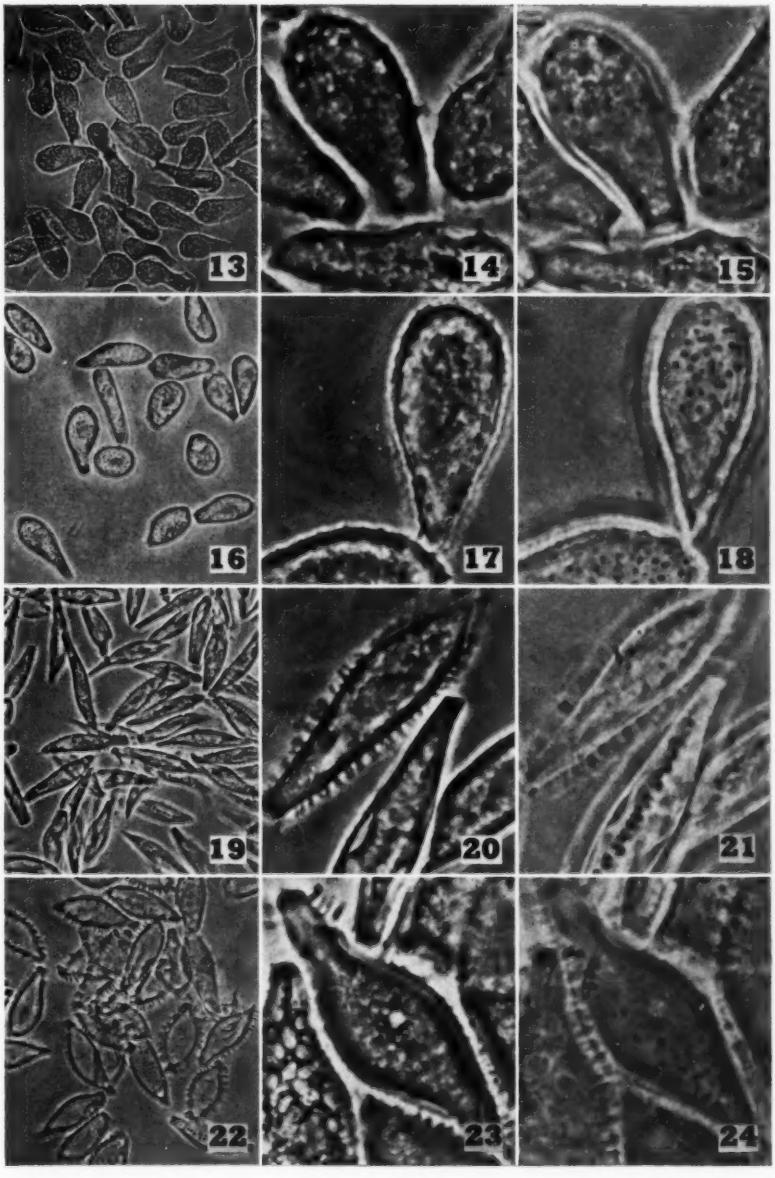
FIGS. 1–6. *Uredinopsis hashiokai* on *Abies grandis*

FIG. 1. Mature pycnium in 1-year-old needle. ×200
FIG. 2. Fresh pycniospores mounted in pycnial exudate. ×1000
FIG. 3. Aeciospores, median optical section. Note the smooth spots. ×1000
FIG. 4. Surface of the spores shown in Fig. 3. ×1000
FIG. 5. Peridial cells, median optical section. ×1000
FIG. 6. Inner surface of the peridial cells shown in Fig. 5. ×1000



FIGS. 7–12. *Uredinopsis pteridis* on *Abies grandis*

FIG. 7. Mature pycnium in 2-month-old needle. ×200
FIG. 8. Fresh pycniospores mounted in pycnial exudate. ×1000
FIG. 9. Aeciospores, median optical section. Note the smooth spots. ×1000
FIG. 10. Surface of the spores shown in Fig. 9. ×1000
FIG. 11. Peridial cells, median optical section. ×1000
FIG. 12. Inner surface of the peridial cells shown in Fig. 11. ×1000



Urediniospores of *Uredinopsis hashiokai* (Figs. 13–18) and *U. pteridis* (Figs. 19–24)

FIGS. 13–15. From the type of *U. hashiokai*. FIGS. 16–18. From expt. E. FIGS. 19–21. Long-spored form of *U. pteridis*. FIGS. 22–24. Short-spored form of *U. pteridis*. FIGS. 13, 16, 19, and 22, ×250, all others ×1000.

Habitat: pycnia and aecia on needles of *Abies grandis* (Dougl.) Lindl. and *A. lasiocarpa* (Hook.) Nutt. (inoculation) in coastal British Columbia, Canada; uredinia and telia on *Pteridium aquilinum* (L.) Kühn var.[4] on the Island of Formosa; on *P. aquilinum* var. *lanuginosum* (Bong.) Fern. at Hawaii (4, p. 80), and coastal California, Oregon, and British Columbia.

Specimens Examined

On *Abies grandis*. BRITISH COLUMBIA: May 10, 1952, 7668[5] (inoculum for expt. A); June 18, 1954, 9210 (inoculum for expt. B); June 8, 1955, 9383, DAOM 58035, PUR 55838, TRTC (inoculum for expt. D); June 8, 1955, 9382, DAOM 58034, PUR 55839, TRTC (inoculum for expt. E); May 23, 1956 9909 (obtained by inoculation in expt. C and used as inoculum in expt. G); July 11, 1955, 9458; July 14, 1955, 9463; Feb. 23, 1956, 9873; May 17, 1957, 10346; May 19, 1957, 10345 (the last five specimens obtained by inoculation in expt. C); May 17, 1957, 10339 (obtained by inoculation in expt. F).

On *A. lasiocarpa*. BRITISH COLUMBIA: May 17, 1957, 10343 (obtained by inoculation in expt. F).

On *Pteridium aquilinum* var. FORMOSA: Nov. 6, 1932, Herb. Hiratsuka (coll. at Mt. Arisan by Y. Hashioka, No. 116, *type*); July 29, 1934, Herb. Hiratsuka (coll. at Mt. Nankotaizan by Y. Hashioka).

On *P. aquilinum* var. *lanuginosum*. CALIFORNIA: Jan. 25, 1893, Herb. Faull No. 11210 (coll. at Pasadena by A. J. McClatchie, type of *Uredinopsis aspera*); Oct. 5, 1908, Barth. Fung. Columb. 2988 (exsiccati); Aug. 11, 1915, Barth. Fung. Columb. 4787 (exsiccati); Aug. 11, 1915, Barth. N. Am. Ured. 1682 (exsiccati). OREGON: Sept. 1909, Herb. H. S. Jackson; Sept. 1909, Herb. H. S. Jackson; July 1910, Herb. H. S. Jackson; July 1910, Herb. H. S. Jackson; Aug. 1910, Herb. H. S. Jackson; Aug. 1910, Herb. H. S. Jackson; Oct. 1914, Herb. H. S. Jackson (the last seven specimens were collected at Corvallis and misidentified as *U. pteridis* D. & H.). BRITISH COLUMBIA: June 30, 1948, 3213; Sept. 10, 1950, 6897; Sept. 10, 1950, 6381, Sept. 3, 1952, 8033; Sept. 9, 1952, 8061; July 5, 1954, 9253; Oct. 12, 1955, 9860; Sept. 5, 1957, 10572; Sept. 5, 1957, 10574; Sept. 5, 1957, 10573; Aug. 22, 1951, 7039; Sept. 26, 1951, 7159; Sept.1, 1952, 8015; Oct. 1, 1952, 8250; Aug. 29, 1955, 9773; July 9, 1954, 9233; July 13, 1954, 9236; July 30, 1954, 9293 (the last three specimens were obtained by inoculation in expt. B, and the last eight specimens contain an admixture of *U. pteridis*); Aug. 10, 1955, 9493; June 8, 1955, 9424; July 2, 1956, 9953; June 9, 1952, 7844; June 1, 1952, 7965 (obtained by inoculation with aeciospores from 2-year-old needles in expt. A); June 1, 1952, 7974 (obtained by inoculation with aeciospores from 1-year-old needles in expt. A); July 9, 1954, 9234 (obtained by inoculation in expt. B); May 24, 1955, 9379 (obtained by inoculation in expt. B and used as inoculum in expt. C); July 4, 1955, 9452; July 14, 1955, 9464, DAOM 58037, PUR 55841, TRTC; Oct. 13, 1955, 9758 (the last three specimens including three duplicates were obtained by inoculation in expt. D); July 4, 1955, 9453 (obtained by inoculation in expt. E); July 14, 1955, 9465, DAOM 58038, PUR 55840, TRTC (obtained by inoculation in expt. E); Oct. 13, 1955, 9926 (obtained by inoculation in expt. D and used as inoculum in expt. F); June 23, 1956, 9945 (obtained by inoculation in expt. G); Sept. 20, 1956, 10015 (obtained by inoculation in expt. G); Sept. 5, 1912, Barth. N. Am. Ured. 881 (exsiccati); Sept. 5, 1912, Barth. Fung. Columb. 4087 (exsiccati).

From examination of over 80 specimens of *U. pteridis* and from observations made in expts. H to M the following additions or changes are suggested to the description of *U. pteridis* (*U. macrosperma* (Cooke) Magn.) published by Faull (4):

UREDINOPSIS PTERIDIS Diet. & Holw., 1895. O, I, II, and III.

Uredinopsis macrosperma (Cooke) Magn., 1904. II.

Pycnia (Fig. 7) on needles 1 month to 4 years old, hypophyllous (on *Abies grandis*) or amphigenous (on *A. lasiocarpa*) . . . Pycniospores (Fig. 8) 1.5–2.5 × 4–6.5 (7.0) μ, straight, cylindrical, hyaline.

[4]The host variety, given as "var. *lanuginosum* Hook." on Hiratsuka's specimen labels, seems questionable. It has been omitted in Faull (4, p. 82).

[5]Numbers following dates are accession numbers of specimens deposited in herbarium DAVFP (10).

Aecia on needles 10 months to 4 years old and hypophyllous (on *A. grandis*), or 4 months old and older and amphigenous (on *A. lasiocarpa*) . . . Peridium laterally dehiscent, often becoming lacerate . . .

Walls of urediniospores (Figs. 19–24) smooth or sparsely verrucose-echinulate, always with two opposing, vertical rows of cogs, the rows of cogs uniseriate to bi- or multi-seriate and thus becoming less distinct . . .

Specimens Examined

Approximately 60 collections of *U. pteridis* were examined. The following represent inoculation experiments mainly. They were divided and distributed to several herbaria as shown below: May 23, 1955, 9352, DAOM 58032, PUR 55828, TRTC (inoculum, expt. H); July 4, 1955, 9451, DAOM 58036, PUR 55837, TRTC (expt. H); Oct. 10, 1955, 9925, DAOM 58040, PUR 55832, TRTC (expt. H, inoculum for expt. K); May 23, 1955, 9380, DAOM 58033, PUR 55836, TRTC (inoculum, expt. I); June 23, 1956, 9946, DAOM 58042, PUR 55834, TRTC (expt. L); July 2, 1956, 9955, DAOM 58043, PUR 55835, TRTC (from Genoa Bay, see expts. H and I); Sept. 20, 1955, 9927, DAOM 58041, PUR 55833, TRTC (inoculum, expt. J); Sept. 20, 1955, 9760, DAOM 58039, PUR 55831, TRTC (from same collection as 9927); May 17, 1957, 10344, DAOM 58045, PUR 55830, TRTC (expt. J); May 19, 1957, 10342, DAOM 58044, PUR 55829, TRTC (expt. J).

In British Columbia, a long-spored form of *U. pteridis* appears to be geographically distinct from a short-spored form (Figs. 19–24), the latter occurring in coastal regions only. Data available, however, are considered to be insufficient to justify a taxonomic distinction between the two forms.

Until characteristics are discovered by which *U. pteridis* can be distinguished from *U. hashiokai* in the aecial state, it cannot be decided which one of the two species of *Uredinopsis* represents the telial state of *Peridermium pseudo-balsameum*, especially if one takes into consideration that the type locality of *P. pseudo-balsameum*, Eureka, Calif., lies within the geographical ranges of both *Uredinopsis pteridis* and *U. hashiokai*.

Field Observations and Geographical Distribution

Rust on bracken fern has been collected throughout western North America where fir grows in the vicinity of bracken, from central and western British Columbia to southern California. On many occasions the writer collected the white *Peridermium* known as *P. pseudo-balsameum* on fir adjacent to rusted bracken fern. Most frequently the *Peridermium* is found on grand fir, but it has also been collected on amabilis and alpine fir, usually in proximity of the bracken rust. The conspicuous absence of bracken fern rusts on the Queen Charlotte Islands, where fir does not grow naturally, supports the theory that *Uredinopsis hashiokai* and *U. pteridis* cannot survive on fern without completing their life cycles on fir; at least not in regions where the fronds of the fern host die off every winter, thus preventing the survival of the rust in the uredinial state.

U. pteridis and *U. hashiokai* are the only two species of rust known to occur on bracken fern in western North America. After examination of over 140 collections of bracken fern rust, from North and South America and Asia but mainly from the west coast of North America, it appears that *U. hashiokai* is much more restricted in its geographical distribution than *U. pteridis*

(compare distribution of *U. pteridis* in Faull's monograph). In North America, *U. hashiokai* seems to be restricted to the west coast, where it occurs quite frequently; approximately one third of the bracken fern collections from southern Vancouver Island and Oregon examined were *U. hashiokai*, the remainder *U. pteridis*. Very few collections contained both species, although *U. pteridis* occurs throughout the known geographical range of *U. hashiokai* in North America.

Life History

The results of 14 inoculation experiments and observations of all spore states on the aecial and telial hosts in the field suggest the following life history for *U. hashiokai* and for *U. pteridis* (Fig. 25):

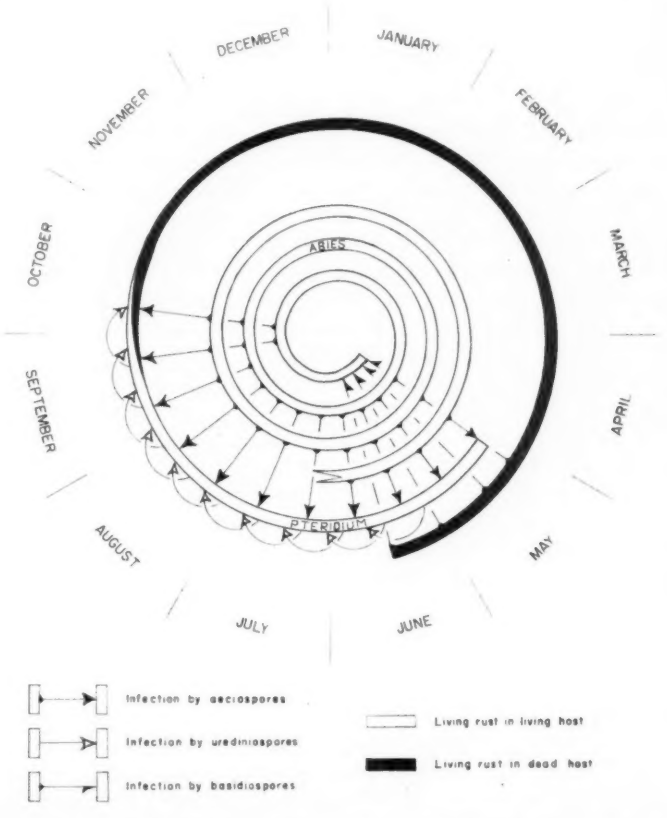


FIG. 25. Diagram of the life cycle of *Uredinopsis hashiokai* and *U. pteridis* from observations made near Victoria, B. C.

In May and June, the young needles of grand fir are infected by basidiospores from the telia of overwintered fern fronds. Approximately one month later, pycnia with pycnial drops appear on the infected needles. No further symptoms, except a slight discoloration or even death of a few severely infected needles, are noticeable during the remainder of the same growing season. In February of the following year, aecial bulges become visible on the lower surface of the needles, the pycnia of which had been previously spermatized. The aecia eventually break through the epidermis, and sporulation by lateral dehiscence of the peridia begins in March or April, usually before the young fern fronds have unrolled. Spore production and growth of the aecial horns is most rapid in spring, gradually decreases towards late summer and fall, and stops during the winter months when the rust mycelium is dormant. Every spring thereafter, growth and sporulation is resumed in the same manner and by the same aecia, until after 3 to 4 years the infected needles finally die. No new pycnia or aecia are produced on needles 1 year old or older. The infection is perennial, but remains localized. Extreme tolerance of the host is indicated by the almost normal appearance and life span of the infected needles.

Initial infection of the bracken fern by aeciospores begins in spring, as soon as the young fronds have unrolled. The uredinia mature 3 weeks later and the rust becomes progressively abundant on fern during the summer from infections by both aeciospores and urediniospores. By fall, telia have developed, which will overwinter in the dead fronds. The teliospores germinate in the following spring. With the infection of the young fir needles by basidiospores from the overwintered fern, the 2-year life cycle of the rusts is completed.

Observations indicate that the aecia on artificially inoculated alpine fir mature 5 to 6 months earlier than on grand fir, and that the life cycle of both *U. hashiokai* and *U. pteridis* may be completed on alpine fir and fern in 1 year, at least under greenhouse environment (expts. F, J, and M). Furthermore, expts. J and K suggest that alpine fir is less susceptible to the coast form of *U. pteridis* (Figs. 22–24) than to the interior form of *U. pteridis* (Figs. 19–21); no infection was obtained on alpine fir with the coast form of *U. pteridis* in expt. K.

The life history of *U. hashiokai* and *U. pteridis* is outstanding in several respects: (*a*) the periods for development of the pycnia (21–25 days) and aecia (106–358 days) are much longer than in any other species of *Uredinopsis* (4, 5) or *Milesia* (2, 3) studied, with the exception only of *M. pycnograndis* Arth. (*M. polypodophila* Faull (1)), the aecia of which mature in 2 years; (*b*) *M. pycnograndis*, *Uredinopsis hashiokai*, and *U. pteridis* are the only white-spored rusts now known to produce aecia from a perennial mycelium: in the former the mycelium is systemic, whereas in the latter two species it remains localized; (*c*) absence of amphispores, the morphology of the pycnia (Fig. 1) and urediniospores, as well as the exceptionally slow development of the pycnia and aecia suggest phylogenetic affinity of *U. hashiokai* to the genus *Milesia*.

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STUDIES OF WESTERN TREE RUSTS

V. THE RUSTS OF HEMLOCK AND FIR CAUSED BY MELAMPSORA EPITEA[1]

W. G. ZILLER[2]

Abstract

Results from inoculation experiments have shown that the needle rust of hemlock (*Tsuga* spp.), *Caeoma dubium* C. A. Ludwig, represents the aecial state of a new race of *Melampsora epitea* Thuem. for which the name *M. epitea* f. sp. *tsugae* f. sp. nov. is proposed. This race differs morphologically from most species of *Melampsora* and from some races of *M. epitea*. It is indistinguishable, however, from the fir rust *M. abieti-capraearum* Tub. (another race of *M. epitea*), from which it differs in its aecial hosts only. An account of the taxonomy, morphology, and life history of *M. epitea* f. sp. *tsugae* is presented for the first time.

Introduction

The life history of *Melampsora abieti-capraearum*, a needle rust of fir (*Abies* spp.), has been known since 1902, when Tubeuf infected willow (*Salix* spp.) with aeciospores from fir in Bavaria, Germany (14). Since then, Mayor (12), Fraser (4, 5), Bethel (1, 15), and Weir and Hubert (15) have confirmed Tubeuf's results by infecting fir with basidiospores from the telia produced on willow as well as by infecting willow with aeciospores from the caeomata produced on fir. In North America, this needle rust was first known as *Melampsora americana* Arth., then as *M. humboldtiana* Speg., until Arthur took up Tubeuf's name, *M. abieti-capraearum*, in his Manual (2) and reduced the former names to synonymy. The rust has often been confused with *M. arctica* Rostr., which bears its aecia on Saxifragaceae (1, 2). Since *M. abieti-capraearum* was found to be indistinguishable from several other species of *Melampsora* in the uredinial and telial states on willow, Hylander, Jørstad, and Nannfeldt (8) relegated the species to the rank of "race" or "race group" of *M. epitea*. Although the authors did not make the formal transfer from species to race (*forma specialis*), they nevertheless listed *M. abieti-capraearum* as a "race" of *M. epitea*.

In 1936, Hunter (7) described a new species of *Caeoma*, *C. faulliana* Hunter, which was collected on needles of alpine fir (*Abies lasiocarpa* (Hook.) Nutt.) near Banff, Alberta, and which differs from *Melampsora abieti-capraearum* in the morphology of its pycnia. Dr. Hunter remarked: "No doubt it is a species of *Melampsora*; but until someone makes appropriate cultures the identity of its diploid phase will remain unknown".

Caeoma dubium (11), a needle rust of hemlock (*Tsuga* spp.) in western North America, was described in 1915, but its life history has remained

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[2]Forest Biology Laboratory, Victoria, B.C.

unknown. Based on morphological studies and the results of inoculation experiments completed recently, new evidence of the life history of *C. dubium* and its taxonomic relation to *Melampsora abieti-capraearum* and *Caeoma faulliana* has been gained. This evidence is presented herein.

Inoculations

It was assumed a priori that *C. dubium* would be the aecial state of a *Melampsora* which bears its telia on willow: all species that have in their cycle a caeoma state on conifers belong to heteroecious species of *Melampsora*, and, in North America at least, their alternate hosts are either willow or poplar (*Populus* spp.). During a brief microscopic examination it was noted, perhaps for the first time, that the heteroecious species of *Melampsora* which produce caeomata on conifer needles in North America fall into two morphologically distinct groups: the poplar rusts, which are characterized by bilateral thickening of the walls of the aeciospores as well as the urediniospores (Figs. 3, 6, 7, and 10), and the willow rusts, in which bilateral thickening of the spore walls is totally lacking. In the past, this characteristic thickening of the spore walls had been noted only in the urediniospores of poplar rusts. Lack of bilateral thickening of the aeciospore walls indicated, therefore, that willow rather than poplar would be the telial host of *Caeoma dubium*.

The inoculation experiments, summarized in Table I and annotated below, were designed:

(*a*) to compare the life histories of *C. dubium*, the hemlock rust, and *Melampsora abieti-capraearum*, the fir rust (expts. A–L);

(*b*) to obtain specimens of the uredinial and telial states by inoculation with aeciospores of *Caeoma dubium* (expts. A, C, E, and H);

(*c*) to determine whether basidiospores of both, the hemlock rust and the fir rust, are capable of infecting conifers of both genera, *Tsuga* and *Abies* (expts. E–L).

Experiments A and B

Caeoma dubium was collected near Lake Cowichan, approximately 40 miles northwest of Victoria, and used as inoculum for expt. A. Part of the infections obtained on willow (*Salix scouleriana* Barr. f. *poikila* Schneid.) were harvested after the leaves had died in October; the leaves were overwintered outdoors and used in the following spring as inoculum for expt. B. Most of the needles of western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) infected in expt. B produced aecia. Drops of exudate were visible on the pycnia before the aecia were formed. Maturation of the aecia of *Caeoma dubium* marked the end of the life cycle of the hemlock rust.

Experiment C

Experiment C is a repetition of expt. A: the source of inoculum, place of inoculation, the host inoculated, and the results obtained were almost identical in both experiments.

TABLE I

Inoculation experiments with *Melampsora epitea*

Expt.	Inoculum	Host inoculated, location	Date of inoculation	No. of days to appearance of: 0	I	II
		Inoculations with *Caeoma dubium*				
A	I from *T. heterophylla* field collection	*S. scouleriana* forma *poikila*, f	July 5, 1951			19
B	IV from *S. scouleriana* f. *poikila*, from expt. A	*T. heterophylla*, g	May 27, 1952	19	33	
C	I from *T. heterophylla* field collection	*S. scouleriana* forma *poikila*, f	June 22, 1952			22
D	IV from *Salix* sp. field collection	*P. menziesii*, g	Apr. 28, 1953	—	—	
		L. occidentalis, g	Apr. 28, 1953	—	—	
		A. grandis, g	Apr. 28, 1953	—	—	
		T. heterophylla, g	Apr. 28, 1953	25	31	
		T. mertensiana, g	Apr. 28, 1953	25	31	
E	I from *T. heterophylla* field collection	*S. sitchensis*, g	July 5, 1955			22
		S. scouleriana forma *poikila*, f	July 6, 1955			13
F	IV from *S. sitchensis*, from expt. E	*A. grandis*, g	May 7, 1956	—	—	
		A. lasiocarpa, g	May 7, 1956	—	—	
		T. heterophylla, g	May 11, 1956	11	17	
G	IV from *S. scouleriana* f. *poikila*, from expt. E., type of *M. epitea* f. sp. *tsugae* f. sp. nov.	*A. grandis*, g	May 7, 1956	—	—	
		A. lasiocarpa, g	May 7, 1956	—	—	
		T. heterophylla, g	May 11, 1956	11	17	
		T. heterophylla, f	May 13, 1956	12	17	
		T. mertensiana, g	May 11, 1956	11	19	
H	I from *T. heterophylla*, from expt. G	*S. scouleriana* forma *poikila*, f	June 1, 1956			14
		Inoculations with *Melampsora americana* (*M. abieti-capraearum*)				
I	I from *A. grandis* field collection	*S. scouleriana* var. *thompsoni*, f	July 4, 1955			15
		S. scouleriana forma *poikila*, f	July 4, 1955			15
J	IV from *S. scouleriana* var. *thompsoni*, from expt. I	*A. grandis*, g	May 7, 1956	12	21	
		A. lasiocarpa, g	May 7, 1956	12	21	
		A. amabilis, g	May 7, 1956	?	16	
		T. heterophylla, g	May 11, 1956	—	—	
		T. heterophylla, f	May 13, 1956	—	—	
		T. mertensiana, g	May 11, 1956	—	—	
K	IV from *S. scouleriana* forma *poikila*, from expt. I	*A. grandis*, g	May 7, 1956	12	21	
		A. lasiocarpa, g	May 7, 1956	15	21	
		T. heterophylla, g	May 11, 1956	—	—	
		T. heterophylla, f	May 13, 1956	—	—	
		T. mertensiana, g	May 11, 1956	—	—	
L	I from *A. grandis*, from expt. K	*S. scouleriana* forma *poikila*, f	June 1, 1956			14

NOTE: *A.* = *Abies*; *L.* = *Larix*; *P.* = *Pseudotsuga*; *T.* = *Tsuga*; *S.* = *Salix*.
The Roman numerals 0–IV denote the *mature* pycnial, aecial, uredinial, telial, and basidial spore states, respectively.
f (field) and g (greenhouse) denote the place of inoculation.

Experiment D

The inoculum consisted of dead willow leaves collected at the foot of Mt. Arrowsmith, Vancouver Island, in November, 1952. The willow grew among young hemlock which had been found to be rusted with *C. dubium* 3 months earlier. No fir was seen in the vicinity of the willows. Microscopic examination showed that the willow leaves used as inoculum contained telia and old uredinia of *Melampsora epitea*.

Western hemlock and mountain hemlock (*Tsuga mertensiana* (Bong.) Carr.) became infected with *Caeoma dubium*, while Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco), western larch (*Larix occidentalis* Nutt.), and grand fir (*Abies grandis* (Dougl.) Lindl.) as well as all controls remained free of rust. The relatively long period required for maturation of the pycnia and aecia (compare with expts. F and G) is ascribed to low temperatures prevailing during the experiment. The species of willow which bore the inoculum could not be identified.

Experiments E to L

Experiments A to D had revealed no physiological or morphological difference between the hemlock rust and the fir rust. Experiments E to L were carried out to investigate their host relationships by comparing their life cycles under equal environment.

Experiments E to H.—The initial inoculum for this series of experiments consisted of aeciospores of *Caeoma dubium* from a field collection made near Duncan, Vancouver Island. Leaves of two species of willow, *Salix sitchensis* Sans. and *S. scouleriana* f. *poikila*, were infected in expt. E and used for inocula in expts. F and G. Finally, aeciospores of *Caeoma dubium* obtained by inoculation of western hemlock in expt. G were sown on willow in expt. H, which as a result became amply rusted with uredinia. It is to be noted that fir remained entirely free of rust (expts. F and G).

Experiments I to L.—The initial inoculum for expts. I to L consisted of aeciospores of *Melampsora abieti-capraearum* from a field collection made at a location near Victoria where no hemlock occurred. Amabilis fir (*Abies amabilis* (Dougl.) Forb.) and a variety of Scouler willow (*Salix scouleriana* var. *thompsoni* Ball) were included, and Sitka willow (*S. sitchensis* Sans.) was omitted as test plants, but otherwise the materials and procedures for expts. I to L were the same as for expts. E to H, above. It is to be noted that hemlock remained entirely free of rust in expts. J and K, but that both the fir rust and the hemlock rust caused uredinial infection at the same date, in equal abundance, and on the same willow tree in expt. L and in expt. H.

The methods used in this study were essentially the same as outlined in a previous publication (17). All controls remained free of rust. Inoculations were performed in an unheated and partially shaded greenhouse. Artificial spermatization of the pycnia (see ref. 17) was not carried out. The following photomicrographs are representative of the inoculation material used: Fig. 1

(expt. E), Fig. 2 (expt. G), Figs. 6–8 (expt. H), Figs. 9 and 10 (expt. E, *Type*), Fig. 11 (expt. I), Figs. 12, 14, and 15 (expt. K), Figs. 16–18 (Expt. L), and Figs. 19 and 20 (expt. I).

One may conclude from the experiments that *S. scouleriana* f. *poikila*, the willow tested, is equally susceptible to both the hemlock rust and the fir rust, and that the two forms of rust are distinguishable from each other by their aecial hosts.

Taxonomy

The results of inoculation experiments have left no doubt that the hemlock rust known as *Caeoma dubium* in its aecial state completes its life cycle as a leaf rust of willow. In deciding its taxonomic position and the name of its perfect state the writer has followed Jørstad's classification of the willow rusts, which received wide recognition (3, 8, 13, 16). Jørstad includes several willow rusts "usually looked upon as separate species" under *Melampsora epitea* (9). He discusses in some detail the taxonomic relationship of the species he includes and summarizes his concept of *M. epitea* as follows:

"Under this collective name are here placed various races and race groups of *Salix* rusts that are usually looked upon as separate species, chiefly on the strength of possessing different haplont hosts; they are all characterized by subepidermal teleutosori with the spores not thickened apically, by thick-walled inner uredoparaphyses, and roundish or oval, evenly echinulate uredospores. Independence of host-alternation is common within these rusts, owing to hibernating diploid mycelium probably chiefly in buds" (9, p. 105).

If Jørstad's concept is followed, one must include the hemlock rust under *Melampsora epitea*. It remains to be decided, however, whether the hemlock rust should be submerged in one of Jørstad's subspecific taxa of *M. epitea* or be segregated as a new and distinct taxonomic entity.

To determine its taxonomic position more precisely, specimens of the following species of *Melampsora* and *Caeoma* were examined and compared with the hemlock rust (*Melampsora epitea*): *M. abietis-canadensis* C. A. Ludwig ex Arth., *M. ribesii-purpureae* Kleb., *M. arctica* Rostr., *M. paradoxa* Diet. & Holw., *M. abieti-capraearum* Tub., and *Caeoma faulliana* Hunter. The comparison showed that each of the species examined, except *Melampsora abieti-capraearum*, differs distinctly from the hemlock rust in the following morphological characteristics:

M. abietis-canadensis: bilateral thickening of the walls of the aeciospores and urediniospores, a general characteristic of the poplar rusts of North America (see first paragraph of section Inoculations); aeciospore walls lacking apical thickening; differences in pycnia, aeciospore wall projections, and teliospores.

M. ribesii-purpureae: aeciospore walls lacking apical thickening; aeciospore wall projections rounded-conical instead of rounded-cylindrical, more densely spaced; urediniospore wall thicker and often angled.

M. arctica: aeciospores much larger; aeciospore walls lacking apical thickening; aeciospore wall projections more densely spaced. It is to be noted

that before the year 1920 *M. abieti-capraearum* (= *M. americana*) had been studied under the erroneous name of *M. arctica* Rostr. This misapplication of the latter name was later corrected by Arthur (1, p. 468).

M. paradoxa (= *M. bigelowii* Thuem., ref. 3): pycnia smaller, subcuticular; wall of mature aeciospores thicker but lacking apical thickening; aeciospore wall projections rounded-conical and more densely spaced; uredinia, urediniospores, telia, and teliospores much larger but very variable in size.

M. abieti-capraearum: morphologically indistinguishable from the hemlock rust (*M. epitea*) in all five spore states. Hunter's studies confirm the similarity of the pycnia of *M. abieti-capraearum* to the pycnia of *Caeoma dubium*: *Melampsora abieti-capraearum* (*M. americana*), pycnia subepidermal, 79–154 μ wide × 42–71 μ high (6, p. 6, Fig. 1; 7, pp. 116–118, Table II, Figs. 1–3), pycniospores 1–1.5 × 3.2–4.5 μ (6, Table I); *Caeoma dubium*, pycnia subepidermal, 80–115 μ wide × 50–90 μ high (7, p. 119), pycniospores 1.5–2×3–4.5 μ (from the writer's diagnosis).

Notes on the dimensions of the spores measured by C. A. Ludwig are enclosed with most of the seven specimens of *Melampsora abieti-capraearum* obtained from the Arthur Herbarium (PUR). Ludwig's measurements are slightly smaller than the measurements obtained by the writer from the same specimens. It is assumed that Ludwig included in his spore mounts small, immature spores, which the writer excluded from his spore mounts.

Apical thickening of the aeciospore walls of *M. abieti-capraearum* was first recorded by Weir and Hubert (15, p. 117) as being a constant characteristic of that species. The writer found it to be a constant characteristic in *Caeoma dubium* as well. Although difficult to recognize, particularly in fresh spores, the thickening becomes more evident in cross sections of young caeomata and in spores which have been boiled in lactophenol.

Caeoma faulliana: pycnia subcuticular, pustulate, about one-half the size of the pycnia of *Melampsora abieti-capraearum* and *Caeoma dubium*. *Melampsora abieti-capraearum* and *Caeoma faulliana* are the only needle rust species forming caeomata on fir known to occur in North America. It seems that *C. faulliana* is represented only by its type on alpine fir, which consists of three permanent mounts of cross and longitudinal sections of alpine fir needles containing approximately fifty pycnia and five caeomata. The measurements of the pycnia of *C. faulliana*, 35–85 μ wide × 20–50 μ high excluding compound and confluent pycnia, were compared with measurements of pycnia of *Melampsora abieti-capraearum* obtained by inoculation of alpine fir in expt. J, 105–140 μ wide × 55–95 μ high. The comparison fully confirms Dr. Hunter's original observations on *Caeoma faulliana*: "The spermogonia . . . at once distinguish this rust from *M. Abieti-Capraearum* which it superficially resembles. They are smaller, more elevated (pustular) and plainly subcuticular. No doubt it is a species of *Melampsora* . . ." (7).

Specimens Examined

Melampsora abietis-canadensis: on *Tsuga canadensis* (L.) Carr., Granville, N.Y., June 23, 1922, H. S. Jackson, TRTC;[3] on *Populus deltoides* Bartr., West Lafayette, Ind., Sept. 19, 1915, H. S. Jackson, TRTC. *Melampsora ribesii-purpureae*: on *Ribes alpinum* L., Norway,

May 31, 1906, O. Dahl, TRTC; on *Salix* sp., New Denver, B.C., July 30, 1930, A. W. McCallum, DAOM 1772. *Melampsora arctica*: on *Leptasea flagellaris* (Willd.) Small, Pikes Peak, Colo., July 8, 1893, J. A. Stevenson, TRTC; on *Saxifraga oppositifolia* L., Norway, 1887, A. Blytt, TRTC. *Melampsora paradoxa*: on *Larix occidentalis* Nutt., Needles, B.C., June 28, 1954, W. G. Ziller, DAVFP 9226; Victoria, B.C., April 13, 1953, W. G. Ziller, DAVFP 8383 (obtained by inoculation in greenhouse with telial inoculum on *Salix lasiandra*); on *Salix lasiandra* Benth., Saanich, V.I., B.C., March 4, 1953, W. G. Ziller, DAVFP 8386 (contains inoculum for DAVFP 8383, above); Saanich, V.I., B.C., Sept. 26, 1951, W. G. Ziller, DAVFP 7144 (from same tree from which the inoculum for DAVFP 8383, above, was collected). *Melampsora abieti-caprаearum*: on *Salix discolor* Muhl., Pictou, Nova Scotia, Nov. 1910, W. P. Fraser, PUR 2851 (Type of *Melampsora americana*, partly used to inoculate *Abies balsamea* (L.) Mill. in the following spring, ref. 4, p. 187); on *Abies balsamea*, Pictou, Nova Scotia, June 10, 1911, W. P. Fraser, PUR 2782 (obtained by inoculation with PUR 2851 above; ref. 4, p. 187); on *A. balsamea*, greenhouse of Macdonald College, Province of Quebec, June 1912, W. P. Fraser, PUR 2783 (obtained by inoculation with telia on *Salix* sp.; ref. 5, p. 238); on *Abies concolor* Lindl., Palmer Lake, Colo., July 15, 1916, E. Bethel, PUR 2790 (used to inoculate three species of *Salix* on July 16, 1916; ref. 1, p. 467); on *S.* (?) *bebbiana* Sarg., Denver, Colo., Aug. 3, 1916, E. Bethel, PUR 2977 (obtained by inoculation with PUR 2790, above; ref. 1, p. 467); on *S.* (?) *scouleriana* Barr., Denver, Colo., Aug. 3, 1916, E. Bethel, PUR 3008 (obtained by inoculation with PUR 2790, above; ref. 1, p. 467); on *S. bebbiana* var. *perrostrata* (Rydb.) Schneid., Denver, Colo., Aug. 20, 1921, E. Bethel, PUR 2989 (obtained by inoculation with aeciospores on *Abies concolor* from Palmer Lake, Colo.); 15 specimens representing the writer's culture material for expts. I–L (Table I). *Caeoma faulliana*: on *Abies lasiocarpa*, Banff, Alberta, July 8, 1925, W. R. Watson, Herb. J. H. Faull No. 8849, now at TRTC (type of *Caeoma faulliana*).

The analysis indicates that the hemlock rust can be distinguished on a morphological basis from the other rusts examined, with the exception of *Melampsora abieti-caprаearum*. Experiments E–L are considered to have proved sufficiently that the hemlock rust can be delimited from *M. abieti-caprаearum* by its aecial hosts. Since the perfect state of the hemlock rust has not been recognized and named before, the following name with description is proposed:

MELAMPSORA EPITEA Thuem., Mitteil. Forstl. Versuchsw. Oesterr. 2, 38 et 40 (1879), f. sp. **tsugae** f. sp. nov.

Caeoma dubium C. A. Ludwig, Phytopathology, 5, 281 (1915).

Pycnia in foliis huius anni, hypophylla (in *Tsuga heterophylla*) vel amphigena (in *T. mertensiana*), epidermide tecta, ellipsoidea, 80–140 μ lat., 120–200 μ long., 50–90 μ alt. Spermatia oblonga, hyalina, 1.5–2 × 3–4.5 (6) μ. Aecidia in foliis huius anni, hypophylla (in *T. heterophylla*) vel amphigena (in *T. mertensiana*), 200–400 μ lat., 200–600 μ long. Aecidiospores subglobosae, 14–21×15–24 μ; parietes sporarum verrucosi, 0.9–1.8 μ crass. verrucis exclusis, parietibus ad summas 2–4 (6) μ crass.; verrucae cylindriformae plus minusve isodiametricae, 0.4–0.8 μ diam., 0.5–1 μ alt., 0.6–1.4 μ a centro ad centrum. Uredinia hypophylla, minuta, epidermide tecta, (40) 50–150 (200) μ diam. Urediniosporae subglobosae usque ad late obovoideae, 12–17 × 14–20 (22) μ; parietes sporarum ab echinulatis subverrucosi, hyalini, aequaliter 0.6–1.0 μ crass. projecturis exclusis; echinulae conoideae, 0.5–0.8 μ diam., 0.5–0.8 μ alt., 1.0–2 μ a centro ad centrum. Telia hypophylla, epidermide tecta, 150–500 μ diam. Teleutosporae 6–14×16–30 μ, parietibus aequaliter 1 μ crass. Basidia clavata 5–8×30–40 μ. Basidiosporae subglobosae, apiculatae, 5–7 (8) μ diam.

Habitat.: in foliis huius anni *Tsugae heterophyllae* (Raf.) Sarg., *T. mertensianae* (Bong.) Carr., *Salicis scoulerianae* Barr. f. *poikilae* Schneid. et *S. sitchensis* Sans. in Alaska, British Columbia, Washington, Oregon, et Idaho boreali.

Pycnia on needles of the current season, hypophyllous (on *Tsuga heterophylla*) or amphigenous (on *T. mertensiana*),[4] scattered among the aecia, honey-yellow to light brown, originating under the epidermis, depressed,

[3]For abbreviations of names of herbaria Lanjouw and Stafleu (10) are followed.

[4]As a rule, fructifications of conifer needle rusts will develop on all surfaces of the needle that bear stomata. Thus pycnia and aecia are mainly hypophyllous on needles of conifers like western hemlock, grand fir, and amabilis fir, but amphigenous on needles of alpine fir, mountain hemlock, and the pines.

elliptical in vertical section, 80–140 μ wide × 120–200 μ long × 50–90 μ high (Fig. 1). Pycniospores guttulate, oblong to ellipsoid, hyaline, 1.5–2.0 × 3.0–4.5 (6.0) μ (Fig. 2).

Aecia on needles of the current season, hypophyllous (on *Tsuga heterophylla*) or amphigenous (on *T. mertensiana*), biseriate, orange-yellow when fresh, on yellowish areas over part or all of the needle, elliptic to oblong and confluent, pulverulent, ruptured epidermis evident, 200–400 μ wide × 200–600 μ long or longer. Aeciospores catenulate, globoid to broadly ovoid and ellipsoid, 14–21 × 15–24 μ (Fig. 3). Aeciospore wall without a smooth spot, verrucose, hyaline; wall thickness excluding warts 0.9–1.8 μ at base and sides, and 2–4 (6) μ at apex of spore (Fig. 4); warts seen in spore surface view more or less isodiametric, round to polygonal, never elongate or confluent, crowded to widely spaced, 0.4–0.8 μ in diameter × 0.6–1.4 μ spacing from center to center (Fig. 5); warts seen in median section of spore cylindrical and rounded at the ends, 0.5–1.0 μ high (Fig. 4).

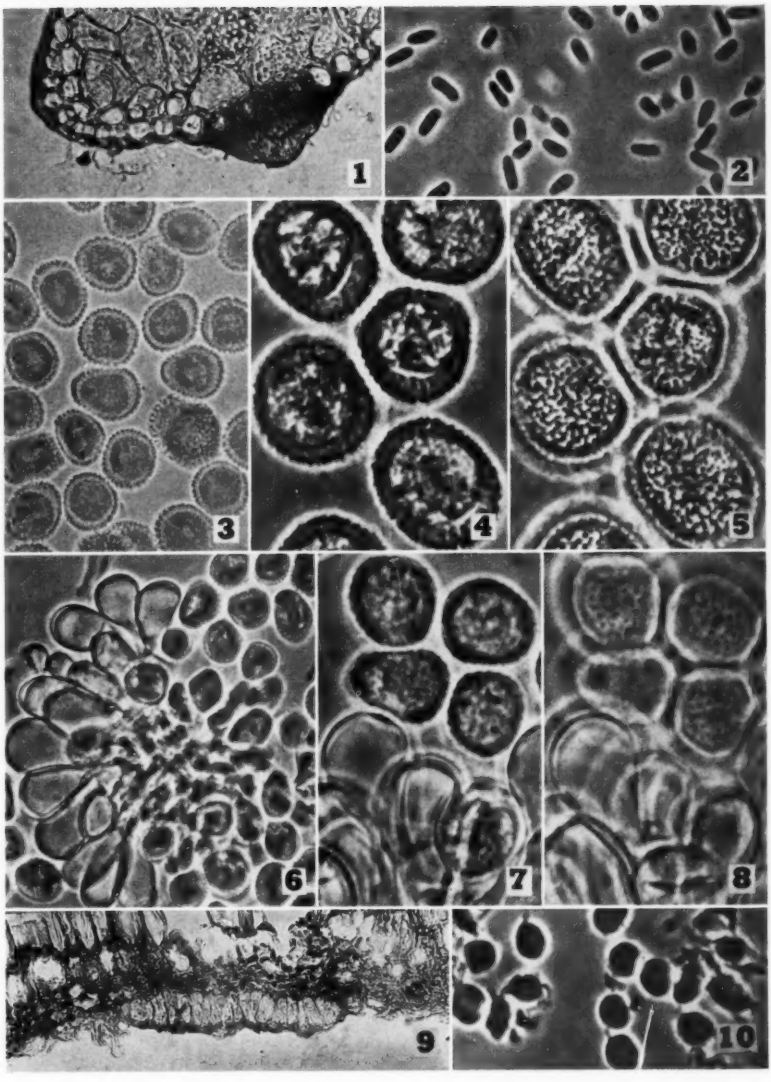
Uredinia hypophyllous, irregularly scattered on yellowed leaf areas, not confluent, lemon-yellow to pale yellow, minute and inconspicuous, round, subepidermal, (40) 50–150 (200) μ (average 70 μ) in diameter. Paraphyses clavate to capitate, 10–18 μ wide × 20–45 μ long; wall of paraphyses smooth, hyaline, either uniformly 0.7–1.5 μ thick or thickened, up to 3 μ, at the apex (Fig. 6). Urediniospores borne on pedicels, globoid to broadly obovoid and ellipsoid, 12–17 × 14–20 (22) μ. Urediniospore wall uniformly verrucose-echinulate, hyaline, evenly 0.6–1.0 μ thick excluding spines; spines conical with rounded points, spaced regularly, 0.5–0.8 μ basal diameter × 0.5–0.8 μ high × 1.0–2.0 μ spacing from center to center (Figs. 7 and 8).

Telia hypophyllous, round to irregularly oblong in surface view, waxy, reddish brown, usually surrounding the uredinia, subepidermal, forming a single layer of spores, 150–500 μ in diameter. Teliospores adhering laterally, prismatic in surface view of telium, oblong and rounded at both ends in cross-sectional view of telium, 6–14 × 16–30 μ; teliospore wall light brown, smooth, uniformly 1 μ thick (Fig. 9). Basidia 4-celled, slightly curved, 5–8 × 30–40 μ; basidiospores light brown in mass, subglobose, apiculate, 5–7 (8) μ in diameter (Fig. 10).

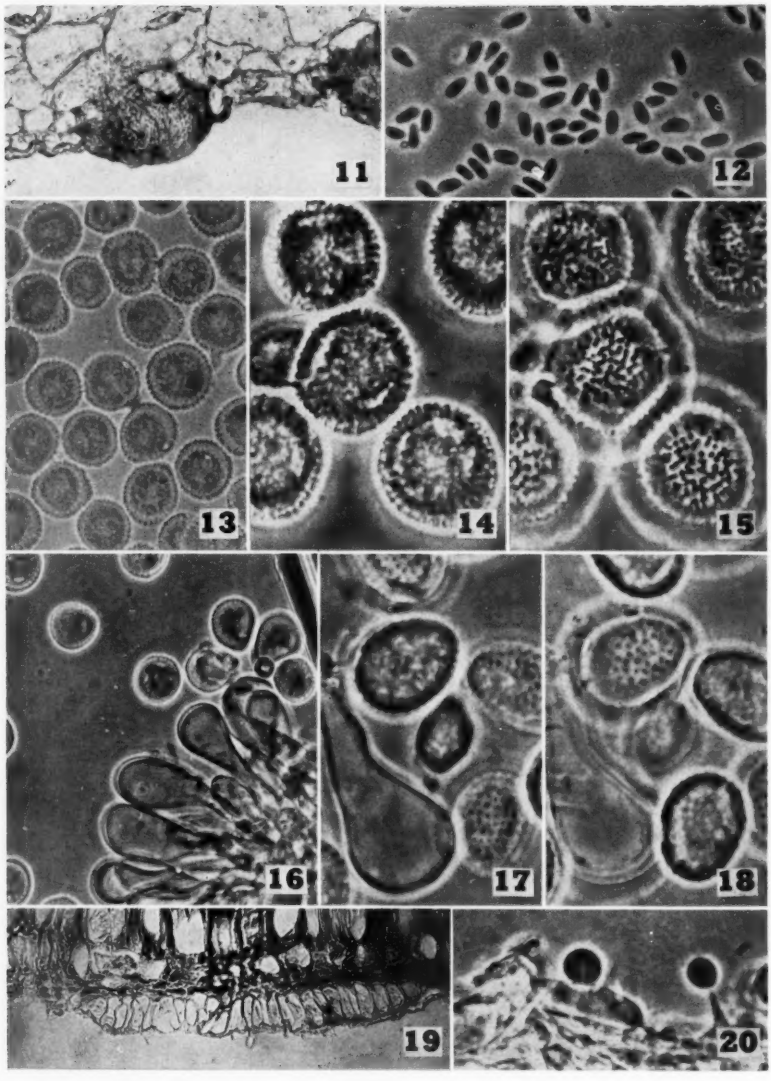
Habitat: pycnia and aecia on current year's needles of *Tsuga heterophylla* (Raf.) Sarg. and *T. mertensiana* (Bong.) Carr. in Alaska, British Columbia, Washington, Oregon, and northern Idaho; uredinia and telia (from inoculation only) on the leaves of *Salix scouleriana* Barr. f. *poikila* Schneid. and *S. sitchensis* Sans. on southern Vancouver Island, British Columbia.

Specimens Examined

The diagnosis is based on 25 specimens obtained by inoculation in expts. A to H; the geographical range has been derived from an additional 28 collections of *Caeoma dubium*, all deposited at herbarium DAVFP. The following list contains only a few of the larger collections obtained by inoculation; they have been divided and distributed among the herbaria listed: On *Tsuga heterophylla*: June 7, 1956, DAVFP 9940, DAOM 58412, PUR 55843, TRTC, obtained in expt. G. On *T. mertensiana*: June 1, 1956, DAVFP 9939, DAOM 58411, PUR 55842, TRTC, obtained in expt. G. On *Salix scouleriana* f. *poikila*: Aug. 24, 1955, DAVFP 9547, DAOM 58403, PUR 55846, TRTC, II,III, obtained in expt. E; Nov. 14, 1955. DAVFP 9627, DAOM 58407, PUR 55847, TRTC, Type, II, III, IV, obtained by inoculation in expt.



FIGS. 1–10. *Melampsora epitea* f. sp. *tsugae*.
FIG. 1. Pycnium, ×200. FIG. 2. Pycniospores, ×1000. FIG. 3. Aeciospores, note apical thickening of spore walls, ×500. FIG. 4. Aeciospores, median optical section, ×1000. FIG. 5. Surface of the spores shown in Fig. 4, ×1000. FIG. 6. Urediniospores and paraphyses, ×500. FIG. 7. Same as Fig. 6 but median optical section, ×1000. FIG. 8. Surface of the spores shown in Fig. 7, ×1000. FIG. 9. Overwintered telium from the type specimen, ×200. FIG. 10. Basidiospores from type, ×1000.



FIGS. 11–20. *Melampsora abieti-capraearum.*

FIG. 11. Pycnium, ×200. FIG. 12. Pycniospores, ×1000. FIG. 13. Aeciospores, note apical thickening of spore walls, ×500. FIG. 14. Aeciospores, median optical section, ×1000. FIG. 15. Surface of the spores shown in Fig. 14, ×1000. FIG. 16. Urediniospores and paraphyses, ×500. FIG. 17. Same as Fig. 16, ×1000. FIG. 18. Same spores as in Fig. 17 but in higher focal plane, ×1000. FIG. 19. Overwintered telium, ×200. FIG. 20. Two basidiospores, ×1000.

E and used as inoculum in expt. G. June 29, 1956, DAVFP 9952, DAOM 58414, PUR 55845, TRTC, II, obtained in expt. H. On *S. sitchensis*: Nov. 12, 1955, DAVFP 9626, DAOM 58406, PUR 55848, TRTC, III, obtained by inoculation in expt. E and used as inoculum in expt. F.

Field Observations

Infections of *Caeoma dubium* have been observed essentially throughout the geographical range of western hemlock during the rust's fruiting period, from the end of May until the middle of August. The infections are usually sparse and restricted to regeneration growth and the lower branches of saplings, the heaviest infection observed by the writer affecting less than 10% of the current year's foliage. The needles become discolored even before the pycnia appear and are shed before the end of the summer. At 12 locations the rust was noted to occur among willows, and at 6 of these locations the willow was found rusted with *Melampsora epitea* later in the season. Very few collections on mountain hemlock have been obtained, chiefly because this host largely occurs on higher elevations and has not been encountered frequently in field sampling.

Two collections on hemlock, one from Skagway, Alaska, collected by D. V. Baxter and the other from Tlell, Queen Charlotte Islands, appear to represent extensions of the known geographical range of *Caeoma dubium*.

Melampsora abieti-capraearum is stated by Arthur (2) to occur in North and South America and in Europe. In British Columbia it occurs at moderate frequency throughout the range of its coniferous hosts. The needles discolor and die after infection, but usually remain attached to the twigs thereafter. On alpine fir the rust has often been collected with *Pucciniastrum epilobii* Otth on the same needles. The 26 aecial collections examined represent *Melampsora abieti-capraearum* on *Abies pectinata* Lam. & DC. from Germany, on *A. balsamea* (L.) Mill. from Wisconsin and Nova Scotia, on *A. concolor* Lindl. from Colorado, on *A. grandis* from Idaho, Washington, and British Columbia, on *A. lasiocarpa* from British Columbia, and on *A. amabilis* obtained by inoculation in expt. J, the latter possibly being the first record of the rust on amabilis fir.

Many field collections of *Melampsora epitea* on willow were examined, but their subspecific identity could not be determined.

Life History

Field observations, confirmed by results from inoculation experiments, indicate the following life history for *Melampsora epitea* f. sp. *tsugae* (Fig. 21):

In May and June, the young needles of hemlock are infected by basidiospores from the telia of overwintered willow leaves. Approximately two weeks later, the infected needles begin to discolor and produce pycnia with pycnial exudate. Aecia mature and begin to shed their spores one to two weeks after the appearance of the pycnia. Aeciospore production continues until August, when most of the aecia-bearing needles have turned yellow and dropped off.

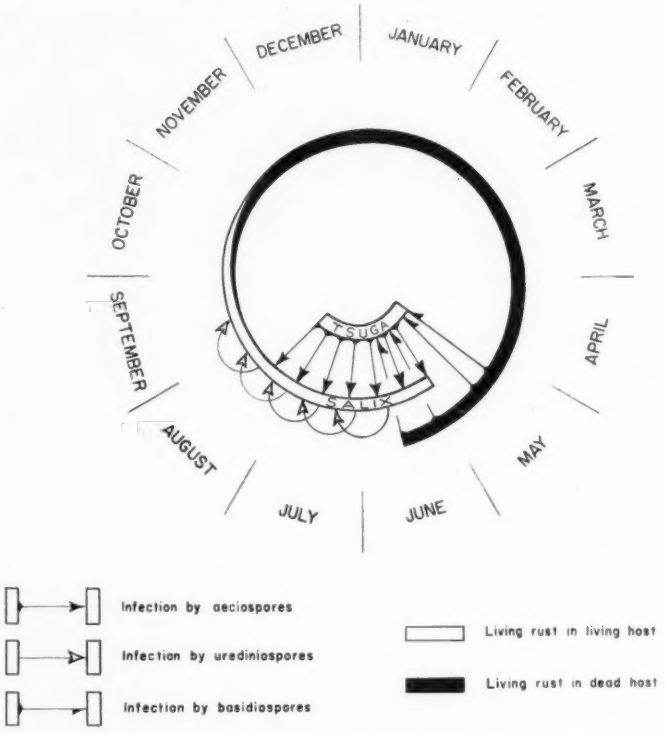


FIG. 21. Diagram of the life cycle of *Melampsora epitea* f. sp. *tsugae*.

Urediniospores are produced on willow leaves approximately two weeks after aeciospore infection. These urediniospores may lead to further infections on willow and the further production of urediniospores, thus intensifying the rust on willow. This intensification may continue until early fall. In fall, telia have developed in the infected willow leaves, which by that time have turned yellow and are shed, usually prematurely, because of the rust infections. The telia overwinter in a state of dormancy in the dead leaves on the ground. Basidia with basidiospores begin to appear on the telia in May, by the time the needles of conifers begin to unfold. The basidiospores once again infect the young needles of hemlock, thus completing the 1-year life cycle of the hemlock rust.

The life history described does not appear to differ significantly from the life histories of other host-alternating melampsoras in North America, including that of *M. abieti-capraearum*. It is not known whether host alternation is obligatory for the hemlock rust or whether *M. epitea* f. sp. *tsugae* can survive without its aecial hosts like several other races of *Melampsora epitea* (8, p. 19 and 13, p. 85).

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CORRELATIONS AFFECTING REGENERATION AND REACTIVATION IN SPLACHNUM AMPULLACEUM (L.) HEDW.[1]

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Abstract

Correlations affecting restitutional behavior in the gametophore are examined. In short stem segments a direct relationship is found between the length of the segment and the number of regenerates; in longer segments this relationship disappears. No such correlation exists between leaf size and number of regenerates per leaf. The presence of leaves increases regeneration from the stem; the stem inhibits regeneration from the leaf.

Isolation and not wounding is shown to be the most important factor in leaf regeneration.

The apex of the gametophore inhibits both regeneration from the base of the gametophore and bud reactivation. These apical dominance effects can be replaced by the application of indoleacetic acid (IAA) to the tips of decapitated plants. Similar concentrations of IAA and γ-phenylbutyric acid (PBA) inhibit regeneration from both isolated stems and leaves; at lower concentrations some promotion is found with IAA, none with PBA. Shorter exposure of leaves to these compounds results in reversible inhibition, or even an increase in regeneration. IAA and PBA do not counteract each other in their effects.

Leaves isolated from intact and decapitated plants show differences in regenerative behavior when treated with IAA. This indicates that IAA could be involved in the inhibition or regeneration from attached leaves.

It is concluded that IAA is not the primary factor in the control of correlative inhibition of restitution in the gametophore.

Introduction

In a recent paper (16) it was shown that correlative factors affect the regenerative behavior in the gametophore leaf in *Splachnum*. A broader study of correlations as they affect the realization of restitutional potentialities in the entire gametophore seemed, however, to be called for. This paper is concerned with an investigation of the correlative factors affecting regeneration and reactivation, the principal restitutional events realizable in the gametophore. A possible mechanism is considered.

It is first necessary to define briefly the terms used in this paper. In plants, structures and activities are interrelated into an organized whole. The organization is such that the plant will react in a specific way to any interference. This reaction has the tendency to re-establish the original constitution, and it is this tendency to maintain the order in the unfolding organization of the whole which is given the general term restitution (6). Restitutional events have largely been classified on a purely phenomenological basis. It seems, however, that a classification on the basis of over-all

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developmental processes should be a more fundamental one. On this basis we distinguish three different types of restitution: *1. regeneration*, *2. reactivation*, and *3. reparation*. The term *regeneration* is restricted to those restitutional events which involve dedifferentiation and redifferentiation. *Reactivation* means release of arrested development of embryonic cells by way of removal of correlative inhibition. *Reparation* finally is that type of restitution involving remolding and growth resulting directly in complete or partial restitution of the original form.

Materials and Methods

The male strain of *Splachnum ampullaceum* was used throughout this study. The plant material originated from the Cambridge, England, Collection. Plants were reproduced vegetatively and grown under strictly sterile conditions on Beijerinck's inorganic solution plus 1% agar. The pH of this medium was adjusted to 6.0. The plant material was grown under continuous white light provided by fluorescent lamps. Gametophores used were always 20–21 days old.

It was necessary for experimental purposes to use isolated plants, and as they usually grow in clusters of four to six, they were isolated by cutting them off at the base. By excising leaves at different time intervals after plant isolation it was shown that there was no decrease in leaf regeneration with gametophore isolation. The isolation treatment did not, therefore, change the regenerative behavior.

Each datum represents the average of at least 12–15 plants or 30–40 leaves. Measurements of protonematal regeneration were made 4 days after experimental treatment. A 1:10,000 solution of neutral red or methylene blue was used to stain the protonematal filaments; this greatly facilitated counting.

Results

Material Size and Regeneration

The relationship between stem size and regeneration was examined by counting the numbers of regenerative protonemata from stem segments of different lengths, stem diameter being considered constant. Figure 1 shows this relationship. In short segments the number of protonemata varied directly with the length of the regenerating stem segment; in longer segments this was not evident.

In contrast to the above, when the numbers of protonemata from isolated leaves were compared with leaf size (Fig. 2) no relationship was found. Coefficients of regression were calculated for several samples; in no case were they significant.

Reciprocal influences between stem size and regeneration from the leaves, and between leaf number and regeneration from the stem, were then examined in small segments. This was done by isolating stem segments of different lengths from the middle portion of the gametophore bearing 0, 1, or 2 leaves.

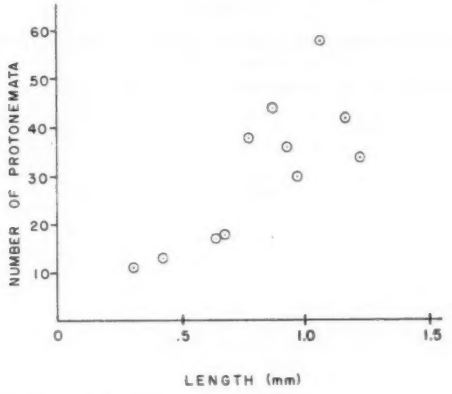

FIG. 1. The relationship between stem length and regeneration per stem segment (short, decapitated stems).

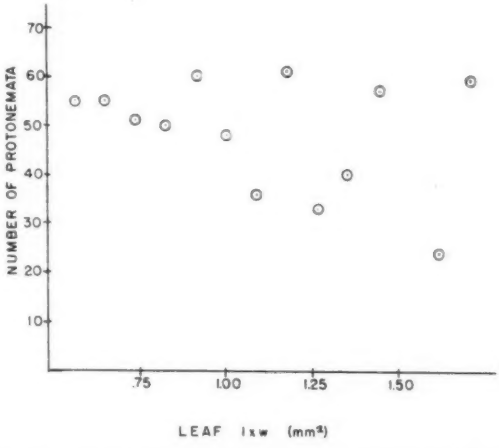

FIG. 2. The relationship between leaf size and regeneration per leaf.

Table I shows the results of this treatment. Stem size in this range had no effect on regeneration from attached leaves; here isolation was the important phenomenon. The presence of a leaf or leaves increased regeneration from the stem; differences between the first corrected value and the second or third are significant.

Wounding and Regeneration

It was next tested whether wounding or isolation was the important factor in the realization of regenerative potentialities. Leaves attached to stems were cut in half at right angles to the longitudinal axis. Following this, the remaining basal half-leaves were either excised or left attached to the

TABLE I

The relationship between stem size and leaf regeneration, and leaf number and stem regeneration

Treatment	Segment length, mm	Number of protonemata		
		From stem		From leaf
		Experimental values	Corrected values*	
Segment and detached leaf	0.60	17.5	21.0	42.0
Segment plus one leaf	0.75	26.0	27.0	1.4
Segment plus two leaves	0.95	34.0	30.0	1.2

*Corrected values: It can be seen from Fig. 2 that in short decapitated stems, regeneration is a linear function of stem length. To correct for differences in length, results were treated as follows: the linearity of the curves for each individual treatment was tested for goodness of fit. If linear, each curve was interpolated to the mean stem length of the entire experiment, and corrected values of protonematal number were read from this point.

plant. Thus comparable material with a large wound surface was obtained, the leaves differing in being isolated or attached to the stem. The numbers of protonemata from these basal half-leaves were counted; Table II gives the results of the experiment. From these data it may be concluded that isolation and not wounding is the important factor in initiating leaf regeneration.

TABLE II

The importance of isolation in initiating regeneration from leaves

Treatment	Number of protonemata
Half-leaves isolated	37
Half-leaves attached	0

The Apex and Regeneration

In higher plants the dominant role of the apical region in the inhibition of restitutional potentialities is well known. It seemed natural, therefore, to investigate the possible effects of the apex on regeneration in *Splachnum* by comparing the behavior of decapitated and intact gametophores. In decapitation the apical region, including the youngest leaves, was removed from the gametophore. The number of regenerates from the stem of the gametophore was determined 4 days after decapitation. The intact gametophore served as the control. Greater regeneration was always found to occur from the stems of decapitated gametophores compared with that from stems of intact plants, the difference varying from 20–100% in different experiments carried out under the same conditions. No significant differences were observed in protonematal length. Results of a typical experiment are shown in Table III.

It was observed that decapitation did not initiate regeneration from leaves attached to the decapitated gametophore. In several experiments comparable leaves from intact and decapitated plants were isolated and planted

TABLE III

Regeneration from intact and decapitated gametophore stems

Treatment	Stem length, mm	Protonematal length, mm	Number of protonemata	
			Experimental	Per mm stem
Intact	1.84	0.82	33	18.2
Decapitated	1.36	0.85	38	28.0

on Beijerinck's medium plus 1% agar. Figure 3 shows the results of these experiments. Differences in the amount of regeneration per leaf between leaves isolated from decapitated and intact plants are not significant. Varying the time interval between decapitation and leaf excision from 0–4 days had no effect.

The possibility that lateral branch apices were replacing the function of the original gametophore apex in decapitated plants was then considered. Segments were isolated and after 5 days any lateral branches which were present were cut off at the base in one group; in a control group they were allowed to grow. Leaves attached to the original stem segments were examined 5 days after this treatment. The following observations were made; as long as a leaf was attached to a healthy stem segment no regeneration had taken place from the leaves, whether lateral branches were present or absent. In some cases the stem segment had died after laterals had begun to grow; here the leaves did regenerate. The presence or absence of lateral branches thus had no visible effect on leaf regeneration.

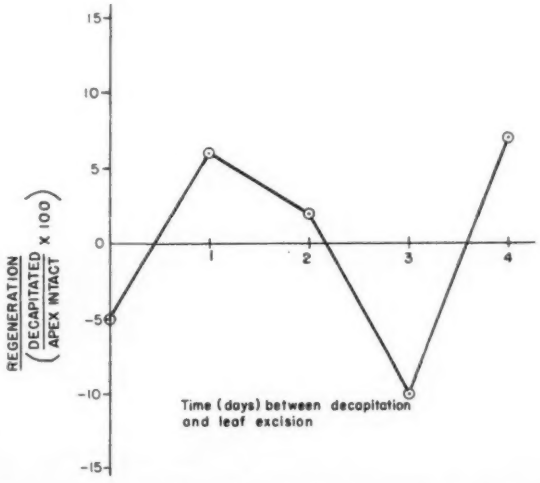

FIG. 3. The effect of gametophore decapitation on regeneration from excised leaves.

The Apex and Reactivation

The possible role of the apex in terms of lateral bud reactivation was examined by comparing lateral bud behavior in decapitated and intact plants. The amount of bud reactivation realized was taken as

$$\frac{\text{number of branches}}{\text{number of axils}} \times 100.$$

Table IV shows the results obtained. Apical dominance in terms of the inhibition of lateral bud reactivation is thus evident. Removal of the apical region results in the removal of influences which inhibit lateral bud reactivation.

TABLE IV

The effect of decapitation on lateral branch reactivation and growth

Treatment	% bud reactivation	Branch length, mm
Intact	29	0.47
Decapitated	97	0.63

The Effect of Auxin and Antiauxin on Regeneration

It is generally assumed that auxin is the limiting factor in the control of reactivation in higher plants. Since the apical region has an effect on restitution in *Splachnum*, indoleacetic acid (IAA) may also be the limiting factor in this correlation system. The effect of concentration ranges of IAA on leaf and stem regeneration was therefore examined. In conjunction with this, the effect of a concentration range of γ-phenylbutyric acid (PBA), an antiauxin, was also tested. Whole plants or isolated leaves were placed on agar containing these compounds at the various concentrations. Figures 4 and 5 show the results of this treatment for regeneration from stems of whole plants and isolated leaves. The curves for IAA and PBA are rather similar, except that IAA promotes leaf regeneration to some extent while PBA does not. Inhibitions are found at high concentrations in all cases. At these higher values the material was often unhealthy or sometimes completely dead.

TABLE V

The reversibility of the inhibition of protonematal regeneration from the leaf caused by IAA and PBA

	Number of protonemata per leaf					
	IAA concn., mg/ml			PBA concn., mg/ml		IAA + PBA, mg/ml
Treatment	0	0.1	1.0	0.1	1.0	0.1 + 0.1
Leaves left in soln.	53	1	0	25	1	0
Leaves removed after 24 hours	50	72	36	59	71	42

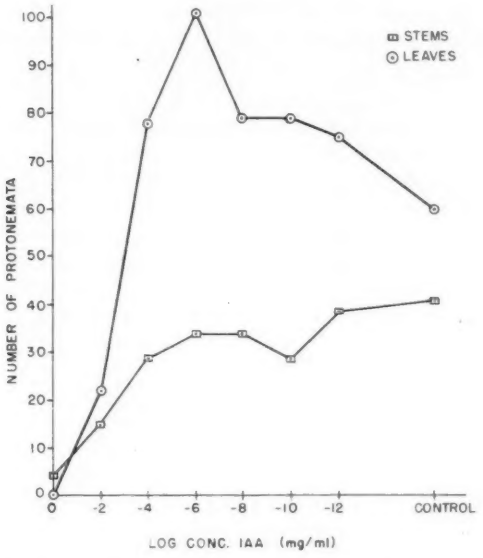

FIG. 4. The effect of IAA on stem and leaf regeneration.

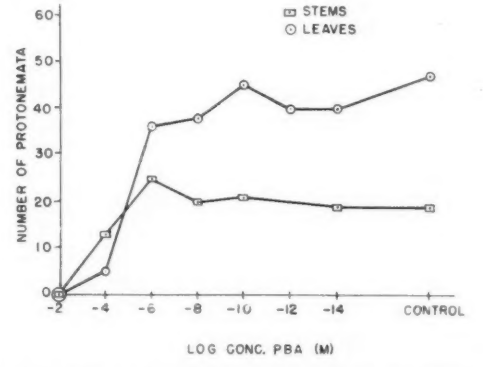

FIG. 5. The effect of PBA on stem and leaf regenerarion.

In conjunction with the preceding experiments, the reversibility of the inhibitions found at high concentrations of IAA or PBA was studied. Leaves were isolated and transferred to IAA, PBA, or mixtures of both. After 24 hours, groups of leaves were removed and transferred to Beijerinck's medium plus 1% agar. The results are described in Table V. As can be seen from this table, the inhibition of regeneration can be reversed within 24 hours. This pretreatment with IAA or PBA actually increased regeneration at certain concentrations; mixtures of IAA and PBA did not counteract each other.

Replacement of the Apical Effect in Regeneration

As the previous experiments showed that IAA had an effect in regeneration, attempts were made to replace the apical effect in decapitated plants by means of IAA. Various concentrations of IAA were made up in 2% agar; small blocks were placed on the tip of decapitated gametophores immediately following decapitation. A plain 2% agar control was used. The blocks were replaced at intervals, usually twice per day, over a 4-day period after which the amount of regeneration was examined. Table VI summarizes the results of a typical experiment. Differences in basal regeneration between apex intact and decapitated plants are again significant. It may be concluded that decapitation increases regeneration from the stem, and that this increase can be inhibited by high concentrations of IAA.

TABLE VI

The effect of apically applied IAA on decapitated stem regeneration

Treatment	Stem length, mm	Number of protonemata	
		Experimental	Per mm stem
Apex intact	1.68	39	23
Decapitated plus plain agar	1.41	49	35
Decapitated plus IAA (1.0 mg/ml)	1.69	30	18
Decapitated plus IAA (0.1 mg/ml)	1.71	52	30
Decapitated plus IAA (10^{-5} mg/ml)	1.39	43	31

As the apex, and IAA, were found to have an effect on stem regeneration, possible influences on leaf regeneration were reconsidered. The hypothesis was considered that a substance such as IAA, produced mainly by the apical region and inhibiting regeneration from attached leaves, was lost so quickly on leaf excision that no differences in regeneration from leaves on intact and decapitated plants were apparent. The fact that the leaves were only one cell in thickness, and diffusion could thus be rapid, made this a distinct possibility. In checking this hypothesis, comparable leaves were again isolated from decapitated and intact plants, the leaves being removed 2 days after decapitation. In this experiment they were transferred to a high concentration (10^{-3} mg/ml) of IAA, instead of to Beijerinck's solution. With reference to Fig. 4, this IAA concentration is on the steepest part of the curve, so that any differences in endogenous IAA should show up with this treatment. Results are shown in Table VII. The number of protonemata from leaves taken from intact plants is significantly less than that from decapitated plants, which is taken as an indication that the IAA level in leaves from decapitated plants is somewhat lower than normal. Therefore IAA could be involved in inhibiting regeneration from attached leaves.

To test this possibility further, the IAA level in the gametophore was raised by application of exogenous IAA to the decapitated tip, and lowered by

TABLE VII

The effect of 10^{-3} mg/ml IAA on regeneration from isolated leaves from intact and decapitated plants

Treatment	Number of protonemata
Leaves from decapitated plants	44
Leaves from intact plants	34

similar application of an antiauxin; 2,3,5-triiodobenzoic acid, which interferes with auxin transport, was used here as the antiauxin. These treatments had no effect on initiating attached leaf regeneration. No significant differences were apparent in regeneration from leaves excised from treated or untreated gametophores.

Replacement of the Apical Effect in Reactivation

Since it was shown that the apical region inhibits bud reactivation correlatively, attempts were made to replace the apical dominance effect in reactivation by means of IAA. The experimental procedure was the same as that in the preceding section. Table VIII shows the results of this treatment. There was again a highly significant increase in the number of branches from decapitated plants. This increase could be inhibited by application of IAA, the amount of inhibition being proportional to the concentration of IAA applied. A relatively high concentration had to be applied to reduce branching to the same order as was found in apex intact plants.

TABLE VIII

The effect of decapitation and IAA application on gametophore bud reactivation

Treatment	% bud reactivation
Intact	12
Decapitated	57
Decapitated plus IAA (10^{-5} mg/ml)	42
Decapitated plus IAA (0.1 mg/ml)	12
Decapitated plus IAA (1.0 mg/ml)	0

Discussion

Interrelationship between the Amount of Regenerating Material and the Amount of Regeneration Realized

If regeneration depends strictly on the amount of regenerating material present, one would expect to find a direct relationship, e.g., between mass and regeneration, surface area and regeneration. If, however, factors of correlation are present, this relationship would not be found.

Loeb (15), studying shoot production from leaves of *Bryophyllum*, stated early that equal masses of new shoots were produced by equal masses of

leaves. From this he concluded that "regeneration" depended on the availability of specific shoot-forming substances in the leaves. The experimental results obtained by several workers (e.g., 7, 9, 19) did not agree with Loeb's theory. Albaum (1), working with *Pteris* prothallia, states that the amount of regeneration depends on the size of the original prothallial segment; this agrees with Loeb's theory. On examining his data, however, this is seen to be not strictly true. Regeneration increases rapidly with increase in prothallial size up to a point; after this the increase becomes less rapid and less linear.

In regeneration from the gametophore stem of *Splachnum*, protonemata seem to be initiated only from the epidermal cells. As the stem diameters are quite constant, one would expect, if Loeb's theory holds, that there would be a linear relationship between gametophore length and number of protonemata. A linear relationship between length of segment and number of regenerates is found in segments up to about 1.2 mm length, in segments longer than this there was no correlation of this type. It is concluded that systems of correlation, which inhibit the realization of regenerative potentialities, are present in the stem, becoming more effective as length increases. These systems are not destroyed by decapitation, and are not important in stem segments less than 1.2 mm in length. Albaum's results could also be interpreted in accordance with this scheme.

As the leaves of *Splachnum* are only one cell thick, regeneration would vary directly with area if correlative factors are not operative. In this study little correlation was found between leaf length $\times$ width and regeneration. It is concluded that systems are present which inhibit regeneration, as has been established by previous workers (5, 8, 16). These factors become more effective with increase in leaf size, so that no particular increase in regeneration with size is found.

Loeb (14) also stated from his work on *Bryophyllum* that if an organ *a* inhibits an organ *b*, then *b* will often accelerate *a*. The present results show that the presence of gametophore leaves increases stem regeneration, while as mentioned above the stems inhibit leaf regeneration. The results discussed in this section do not, therefore, agree with Loeb's mass relationship law, but they do agree with his above interrelationship law.

The Relative Importance of Wounding and Isolation

The objection could be raised that the work of the preceding section does not indicate the presence of correlative factors, but on the contrary only shows that wound phenomena are important in the realization of regenerative potentialities. The experiment with isolated and attached half-leaves was performed to see which was more important in leaf regeneration in *Splachnum*. A large wound surface was present in both treatments. These treatments are not strictly comparable because the isolated group had a larger amount of wound surface, but it is considered that the great difference found in regeneration cannot be ascribed to this quantitative wound difference. Furthermore, when one compares the regenerative behavior of the isolated apical

half of the leaf with the attached basal half, both having the same wound surface, profuse regeneration occurs from the detached apical half and no regeneration from the attached basal half. The total suppression of regeneration in attached half-leaves must have been due to the presence of correlative factors between the leaf and the stem. It is considered that destruction of these correlations, and not wounding, is the important factor in initiating leaf regeneration.

Similar cases have been found in other lower plant forms. For *Splachnum* (16) and other mosses (5, 8, 12), increases of protonematal regeneration have been found with wounding and isolation. Meyer (17) isolated individual cells of the prothallium of the fern *Asplenium adiantum-nigrum* by means of fungal attack, and found that most of the isolated cells would form complete new prothallia. He suggested also that this regeneration is solely due to isolation and consequent destruction of correlative inhibition.

Apical Dominance in Relation to Restitution

Apical dominance phenomena are widespread in higher plants. Cases of apical dominance influencing both reactivation and regeneration have also been reported by a number of workers in lower plants. In ferns, Albaum (2) states that adventitious prothallia arise from the basal portion of *Pteris* prothallia when the apical region is removed. In liverworts, LaRue and Narayanaswami (13) showed that regeneration of adventitious thalli, and germination (reactivation) of gemmae within gemmae cups, are inhibited by the apex in intact thalli of *Lunularia*. In mosses, Kachroo (12), working with sporophytes of *Physocomitrium*, found that the capsule inhibits protonematal regeneration from the seta. Westerdijk (23) showed that protonemata will form from the moss plant only after removal of the apex. Allsopp (3) found that branching in the water fern *Marsilea* was under apical control.

The present study shows a similar apical dominance effect, inhibiting stem regeneration, in the gametophore of *Splachnum*. Removal of the apex increases basal stem regeneration about 35%. In spite of the fact that decapitation did not initiate attached leaf regeneration there is evidence (Table VII) that the apex has some effect here also. Reactivation of lateral branch primordia is inhibited by the apex (Table IV); while intact gametophores at this age showed very little branching, decapitated ones showed much branching. It should be mentioned that apical dominance of both basal regeneration and bud reactivation decreases with increasing age, since in later stages of development of intact gametophores a considerable amount of basal regeneration and lateral branch growth occurs. This is possibly connected with the depletion of the apex during sex organ initiation and development. It may be seen that the following correlation systems are present in the gametophore: (*a*) apex–stem, inhibiting basal regeneration; (*b*) within leaves; (*c*) within stems; (*d*) between stem and leaf; and (*e*) apex–axillary bud, inhibiting its reactivation. These may be various phenomena of one system or of different systems.

The Nature of Correlative Factors Affecting Restitution

One of the most widely accepted theories concerning the mechanism governing correlative inhibition of reactivation in higher plants is a hormonal one. Inhibition of lateral buds was ascribed to the presence in the stem of a concentration of IAA that, while not inhibiting stem growth, inhibited bud development (21). In *Pteris* prothallia Albaum (2) was able to maintain apical dominance after removal of the apical region by the application of IAA to the cut surface. LaRue and Narayanaswami (13) found a similar situation with respect to reactivation of the gemmae in *Lunularia*. Although not all of the experimental data can be explained in these simple terms (e.g. 3, 10, 22), it seemed natural to test the possibility of the presence of an auxin mechanism responsible for the restitutional behavior of the gametophore.

In addition the question arises whether the different types of restitution inhibited by correlative factors are mediated by the same mechanism or whether we may have to assume the presence of separate mechanisms governing regenerative behavior and behavior in terms of reactivation. From certain observations reported here one may assume an identical mechanism; with increase in age of the gametophore apical dominance in terms both of bud reactivation and of basal regeneration decreases. Decapitation of the gametophore resulted in an increase in regeneration. Similarly decapitation brought about the realization of bud reactivation. Application of IAA to the tip of decapitated plants will inhibit both basal regeneration and bud reactivation. These data would indicate, therefore, that at least basal regeneration and bud reactivation may be governed by the same IAA controlled mechanism.

Figures 4 and 5 show the effect of concentration ranges of IAA and PBA on isolated leaf and stem regeneration in *S. ampullaceum*. IAA inhibits leaf regeneration at high concentrations, promotes it at lower ones. Inhibition is found at about the same range in stem regeneration; here no promotion is found. PBA inhibits leaf and stem regeneration at about the same concentrations as IAA. No promotion at all is found here. Several conclusions may be drawn from these experiments. The IAA inhibiting concentration is so high that the total inhibition of regeneration found in attached leaves cannot be due to this compound. Furthermore, leaves and stems seem to have about the same sensitivity to IAA, except that it does not promote stem regeneration. The different patterns of regeneration found in leaves and stems are thus apparently not due to differing sensitivity to IAA. Again, PBA and IAA have similar effects. This indicates an unspecific mode of action of IAA with respect to regeneration.

Skoog *et al.* (20), working with mixtures of IAA and PBA, conclude that PBA acts as an antiauxin by a direct blocking of IAA action; mixtures of IAA and PBA counteract each other. In regeneration in *Splachnum* (Table V) this was not found. In this case mixtures of IAA and PBA had the same effect as the concentrations of IAA or PBA alone; exposure for a short time

increased regeneration, for a longer time caused death. This indicates that certain concentrations of IAA or PBA have a damaging effect on the leaf, which would, if subjected to these concentrations of IAA for a short time, increase regeneration. The mode of action of IAA is still not clear, but one school (4) considers that inhibitions caused by IAA at high concentrations are due to a damage effect.

In the experiments concerning the effect of the apex on isolated leaf regeneration, leaves from apex intact plants regenerated less than the ones from decapitated plants when transferred to IAA. This would indicate that the IAA concentration of leaves from apex intact plants is somewhat higher than that of leaves from decapitated plants. Again there is evidence that IAA could be influential in inhibiting leaf regeneration in normal gametophores. However, leaves attached to decapitated stems did not regenerate (see also 8, 11, 18); here the IAA concentration must have been rather low. IAA cannot therefore be the primary factor involved in the stem–leaf inhibition.

All the correlation systems influencing restitution in *Splachnum* can be replaced by IAA to a greater or lesser extent. From the present data it may be suggested that IAA is involved in restitution and high concentrations are required owing to inactivation of the IAA inside the plant. Alternatively it may be suggested that IAA is not directly involved in restitution, the inhibitions found with applied IAA being due to damage effects. The present discussion, including the data on the similarity of the IAA and PBA effects, would favor the latter suggestion, that the correlative systems inhibiting the realization of restitutional potentialities do not function primarily via an auxin-controlled system, this being particularly true with respect to regeneration.

Wickson and Thimann (24) have recently presented data for apical dominance in *Pisum sativum* which suggest, as stated by them, an interaction between auxin and kinetin-like factor as the mechanism governing reactivation. An investigation of such a mechanism or an even more complicated and less specific kind of interaction may give a clearer picture of the nature of correlations in the gametophore. It may also clarify the question whether the correlation systems governing regeneration and reactivation are identical with or different from each other.

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CYTOGENETICS OF SOLID STEM IN COMMON WHEAT

I. MONOSOMIC F_2 ANALYSIS OF THE VARIETY S-615[1]

RUBY I. LARSON[2]

Abstract

The F_2 lines of monosomics of Chinese Spring by S-615 were compared with normal F_2 lines for stem solidness. The solid stem index of each plant was determined by totalling index numbers (ranging from 1 for thin-walled, hollow, to 5 for completely solid) assigned to cross sections of the culm at seven designated points. As both the normal and monosomic lines showed a continuous distribution of this index, the factorial analysis was not too reliable. The normal F_2 gave an estimated difference between the parents of at least four genes; the monosomic XIII F_2, which was more solid than the normal, gave a difference of three genes.

Quantitative differences between monosomic F_2 lines and the normal indicated that chromosomes II, XIII, and XX, comprising homoeologous group 2, tend to make the culm hollow. Chromosomes XIX and XXI also inhibit pith development. Of these five chromosomes, XIII has a gene for hollow culm in Chinese Spring only; XIX, XX, and XXI, which are in the D genome, are effective in both S-615 and Chinese Spring, and II is inconclusive. Chromosome VIII in Chinese Spring promotes pith production. The chromosomes of S-615 bearing genes for solid stem were not identified by this method, as a monosomic F_2 analysis does not reveal recessives present only in the variety being tested.

Introduction

Varieties of common wheat, *Triticum aestivum* L. *emend* Thell., with pithy culms are more resistant than those with hollow culms to the wheat stem sawfly, *Cephus cinctus* Nort. (8, 9, 12, 21). Rescue and Chinook, the two sawfly resistant varieties, licensed to be grown in Canada, had as their resistant parent S-615, a Portuguese variety obtained by the Experimental Farm at Swift Current from Lincoln College, Christchurch, New Zealand. None of these so-called solid-stemmed varieties is immune to sawfly attack, and none has culms completely filled with pith (23, 24). Although it has not been demonstrated that pith is the only factor that makes a wheat variety resistant to sawfly, when the variety is less solid as a result of environment, it tends to be less resistant. Therefore, an investigation of the genetics of solid stem in S-615 to identify the chromosomes bearing genes affecting pith production was initiated in the hope that this knowledge would improve opportunities of combining these genes with those from other sources.

Literature on inheritance of solid stem indicated that such recombinations might be feasible. Experiments with different varieties and species gave conflicting results, including transgressive segregation in the F_2 generation, indicating that genes affecting solid stem were at several loci. In progeny

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of pentaploid hybrids chromosome number was partly associated with the amount of pith produced in the culm. Aneuploid analysis as developed by Sears (29, 30, 33, 34) offered a promising method of locating genes for solid stem and studying their action.

Literature Review and Basic Hypothesis

Platt *et al.* (22) found that in crosses of S-615 and S-633, solid-stemmed common wheats, with Thatcher and Renown, hollow-stemmed wheats, the F_2 segregated in the ratio of 1 solid to 63 hollow or intermediate, indicating that solid stem is determined by three recessive genes. The F_3 verified this result. Studies on inheritance of solid stem in tetraploid wheats showed solid stem to be due to a single dominant (27), to be due to a single recessive (4), to show transgressive segregation (5), and to be due to complementary recessive genes (6).

In an interspecific triploid cross, Thompson (41) found that the difference between the hollow stem of *T. monococcum* (recessive) and the solid stem of *T. durum* was due to gene differences in the common A genome, not to the B genome possessed by the *durum* only.

Genetic studies of solid stem in hybrids of tetraploids with hexaploids showed that solid stem varied from recessive (2, 10, 11, 39) through intermediate (4, 5, 49) to dominant (44, 45). F_2 segregations were interpreted as monohybrid (2, 4, 5, 10, 11) or multifactorial (44, 45, 49).

Cytogenetic studies of pentaploid hybrids between hollow-stemmed hexaploids and solid-stemmed tetraploids showed that the D genome tends to produce a hollow culm (1, 13, 15, 16, 25, 38, 40, 42, 43, 50), but that differences in solid stem between the two series are partly determined by genes in the A and B genomes (1, 15, 16, 38, 40, 42, 43). A chromosome in the D genome producing hollow stem was identified as *e* (Kihara's nomenclature) = XX (Sears' nomenclature) (17, 18, 53, 54).

Yamashita (52), on the basis of an extensive series of interspecific hybrids between hollow- and solid-stemmed wheats, postulated the following explanation of solid stem inheritance. The A genome has a gene for hollow stem, m_A, that accounts for the hollowness of diploid wheats. This gene has no allele, but is hypostatic to all other genes for solid or hollow stem. The B genome has a gene, M_B, affecting pith content of the stem. It exists as an allelic series, one extreme being represented in the tetraploids that have the pithiest stems and the other in the hexaploid wheats and hollow-stemmed tetraploids. Genes at the M_B locus for solid stem are dominant to their alleles for less solid stem. The D genome has a gene for hollow stem, O_D, that is epistatic to genes for solid stem at other loci. In addition, the A genome has a complementary gene, *C*, for pith in the lower internodes. Although it may be present in hollow-stemmed varieties and species, its effect is not shown in the absence of genes for pith in the B genome.

Aneuploid analysis in common wheat as developed and used by Sears (28, 29, 30, 31, 32, 33, 34, 35, 36) and others (7, 19, 20, 26, 46, 47, 48) has

made it possible to identify chromosomes carrying genes for disease resistance, awn length and hooding, glume color and pubescence, growth habits, and such species differences as speltoidy (chromosome IX), *sphaerococcum* (XVI), and *compactum* (XX). As Sears (33) indicated, monosomics and nullisomics can be used in at least four ways to locate genes for a given character and study their actions and interactions. 1. Direct observation of nullisomics reveals location of dominants acting in the conventional manner, and monosomics reveal recessives. However, dominants that are the residual effects of other genes of the genotype in the absence of the active recessive cannot be located in this way. 2. Disturbed F_2 ratios identify chromosomes carrying simple dominants and the complete coincidence of recessive characters with the disomic condition in F_2 indicates chromosomes bearing recessives. 3. Testing a number of F_3 lines from disomic F_2 plants will reveal the critical chromosome as the one in the descendants of which no segregation for the character occurs. 4. Whole chromosome substitutions of one variety into another make possible intensive studies of the genetic contributions of each chromosome against a uniform genetic background. All methods except the third are being or have been used to analyze solid stem and sawfly resistance derived from the variety S-615.

The research presented in this paper, and previously partly reported in brief (14), concerns a genetic study of monosomic F_2 hybrid populations from the crosses of Chinese Spring plants nullisomic or monosomic for each of the 21 different chromosomes of common wheat with S-615. The assumptions on which it was based are as follows. At the beginning of the investigation, it was accepted that S-615 has three genes for solid stem not possessed by most varieties of common wheat (22). Because Chinese Spring has stems more pithy than most hollow-stemmed common wheats, it was assumed tentatively that it has one gene for pith formation, probably an allele of one of the genes for solid stem in S-615. Gene action was postulated to be the same as that found by Platt *et al.* (22), namely, that only homozygous recessive segregates would be as solid as S-615. A further assumption was made that recessives would not be fully operative in hemizygous condition, that is, when present in single dose as in a monosome. Several recessive genes in wheat have been found to behave in this manner (33). The expected F_2 ratio of normal Chinese Spring × S-615 would be 15 intermediate and hollow to 1 solid. Because environment affects stem solidness, it would be necessary to grow F_3 lines from the solid and near-solid F_2 plants to confirm or correct the F_2 classification. The only monosomic line expected to have a disturbed F_2 ratio would be the one deficient in the chromosome bearing the gene for solid stem common to both parental varieties; it would have a 63:1 ratio instead of the 15:1 in all other lines. The two lines critical for the other two genes would have more near-solid plants than normal and non-critical lines; in them, all fully solid stemmed plants would have 42 chromosomes whereas in all non-critical monosomic lines three quarters of the solid-stemmed plants would be monosomic.

However, early in the investigation it appeared that inheritance of solid stem in this cross was probably not so simple. From a tenth to an eighth of the F_2 population appeared to be as solid as S-615 in 1948, suggesting that perhaps solid stem was at least as complex genetically as awning or head density. If so, the normal F_2 ratio, whatever it be, probably results from the interaction of genes from the two parents, and all plants as solid as S-615 need not necessarily have the same genetic constitution as S-615. Genes affecting pith production might tend to make the stem either solid or hollow, the end result depending on the potency of each gene and its interactions with other genes.

On the above basis different results might be expected to follow loss of critical chromosomes, depending on whether the effective gene was present in Chinese Spring (aneuploid tester), in S-615 (variety being tested), or in both parents. Whatever the effect of the gene, if a Chinese Spring chromosome alone is critical for it, the absence of that chromosome would remove segregation for the gene, that is, it would cut a dihybrid to a monohybrid ratio, a tetrahybrid to a trihybrid ratio. Conversely, if the gene producing solid stem is the same in both parents and is not completely dominant, the number of genes segregating in a critical family would be increased: only the disomics, one fourth of the population, will have the gene in double dose, so there will be only one-quarter as many solid-stemmed plants in the critical family as in others. If, on the other hand, the gene is an inhibitor, is not fully dominant, and is present in only single dose in three quarters of the population (the monosomics), it is hard to predict what deviation from normal to expect except that more of the population would be solid than in non-critical families. If a gene promoting solid stem is in S-615 alone and not fully dominant, there should be no change in the number of solid-stemmed plants, as only disomics would have solid stems and they would constitute a quarter of the population, the same proportion that would be homozygous for that gene in normal and non-critical F_2 lines. The fact that all solid-stemmed plants in the critical family would have 42 chromosomes compared with only a quarter of them in non-critical families provides a clue to the location of the gene on said chromosome. In addition, there would be more nearly solid stemmed plants in the critical family, as three quarters of the population should carry the single gene for solid stem compared with only half of the plants in normal F_2 lines. If a fully dominant gene in S-615 promotes solid stem, the results would be to reduce the number of genes segregating in the critical family by one, and to increase by a third the number of solid-stemmed plants in the population over the normal and non-critical lines.

If S-615 and not the other parent carries a recessive inhibitor of solid stem, the critical line would have the same proportion of solid-stemmed plants as other lines. If the gene produces its full inhibiting effect in a single dose, the number of solid-stemmed plants would decrease by a degree depending on interactions with other genes. It would not entirely eliminate solid-stemmed plants from the population as S-615 itself would be homozygous for the gene

and it is the standard for solid stem index. If the gene inhibits solid stem and has some effect in single dose, but is not completely dominant, the number of solid-stemmed plants would be reduced by a degree that could not be calculated without knowing interactions.

Because of the uncertainty of obtaining reliable Mendelian ratios, it was considered advisable also to study quantitatively the effect of each chromosome on the various populations, that is, whether it generally promoted solid stem or inhibited it. The results expected would be of the same general pattern as those discussed above on the basis of genes and their action. Further, if two genes should be more than 50 crossover units apart, their actions would be independent in the conventional genetic study but they would appear as a unit in populations aneuploid for that particular chromosome.

Although conventional genetic studies reveal gene differences between the parents, they do not indicate the genes for which the parents are alike. Aneuploid analysis of a quantitative character shows whether the various chromosomes are alike or different in the parents, but it does not indicate the number of genes by which they differ. Therefore, there may be a difference between the number of genes estimated on the basis of F_2 ratios and the number of chromosomes apparently revealed by aneuploid analysis. The number of critical chromosomes would tend to exceed the number of genes estimated on the basis of Mendelian ratios in the F_2. This tendency would be reversed only if the genes were sufficiently far apart to segregate independently, or if the gene concerned was completely recessive, was in the non-aneuploid parent only, and only F_2 studies were made.

Materials and Methods

Varieties

S-615

S-615 is a fully-awned, brown-glumed variety of *Triticum aestivum* L. *emend* Thell. The culm is filled or nearly filled with pith at the center of each internode, but there is always a cavity just below each node. The cavity is usually long in the top internode, but is both proportionately and absolutely shorter in the lower internodes. This cavity appears to develop in the same way as the lumen of hollow-stemmed wheats. At the bottom of an internode there is also sometimes a cavity, but it seems to develop because the wall grows faster or longer than the pith. The size of hollow in the stem depends on environment, the amount of sunlight during the growing season being inversely related to the size of hollow (21).

The stock of S-615 was purified by selection for solid stem (22). The uniformity of this stock was shown later by its failure to segregate when selection for solid stem was attempted.

Chinese Spring Aneuploids

Nullisomics I, IV, and VII, and monosomics for all chromosomes but VII, used in these experiments were produced in the variety Chinese Spring by Sears (29, 30, 32, 34). This variety is a spring wheat with a soft, light-red

kernel. It is later than S-615 and tillers more freely. The spike has short top rachis internodes making it "squareheaded". The glumes are light in color and papery in texture, shattering readily. The spikelets are prolific, those in the center of the spike commonly having five or six kernels. The awns are very short and recurved (hooded).

The culm of Chinese Spring is hollow, but it has a thicker lining of pith than the hollow-stemmed varieties of common wheat. It is particularly thick at the top of the peduncle and in the two basal internodes.

The monosomic plants of Chinese Spring are generally not very different from normal in appearance, but those that are resemble the corresponding nullisomic (34). Monosomics of homoeologous group 2 (32, 34), II, XIII, and XX, have short thick culms, broad leaves, thick dense spikes, and deeply cupped glumes and reduced awns. Monosomics VIII and X have longer awns than normal. Monosomic XVI is short and has an extremely clavate spike. Monosomics of homoeologous group 5 have slender culms. Monosomic IX is speltoid. Monosomics V and XVIII have small, slender spikes, and XVIII is very late, producing many leaves before the culm elongates.

Methods—General

Hybridization

Monosomic plants, as determined by chromosome counts of the pollen mother cells, and available nullisomics were emasculated and used as female parents. The cross was made in this direction because about 75% of the female gametes in a monosomic are deficient and functional; only a low percentage of deficient pollen functions owing to competitive disadvantage with normal pollen, although the percentage of deficient pollen is approximately the same as that of egg cells. The source of pollen was the S-615 grown from seed stock as described above. Before a spike was used the culm was cut to see whether it was normally solid because solid stem is recessive and any genetic alternation would be evident.

Growing the F_1

The F_1 populations of nullisomics I and VII and those of certain monosomics that could be distinguished morphologically from normal were grown at Holtville, California, in the winter increase plots of the Cereal Crops Division, Canada Department of Agriculture, during the winter 1947–48. Nulli-IV × S-615 was also grown there in the winter of 1948–49 as this hybrid is almost completely male sterile in the greenhouse, and sets only a few seeds in the field at Lethbridge and Swift Current. Pollen is produced so abundantly in wheat in the Imperial Valley that it was hoped to get a good seed set there. All other F_1 lines were grown in the greenhouse at the University of Missouri, Columbia, Missouri, the Experimental Farm, Swift Current, Saskatchewan, or the Science Service Laboratory, Lethbridge, Alberta, or in field plots at Swift Current or Lethbridge.

The F_2 Tests

Seed from the monosomic F_1 plants was used for monosomic test lines and that from normal F_1 plants for normal F_2 checks. The F_2 tests were grown at Swift Current in 1948 and at Lethbridge in other years. They were tested on dry land, conducive to a high expression of stem solidness. This minimized the number of genetically solid plants that would be classified as not solid because of environmental effects.

Cytology

Chromosome counts were made on the monosomic lines to select parents and on the F_1 plants to identify monosomics. Whole spikes were fixed in Carnoy's fluid, or, when time permitted, individual florets were fixed in Farmer's fluid. Counts were made on temporary acetocarmine squash preparations of pollen mother cells.

Evaluation of Solid Stem

Because solid stem is a quantitative character, any method used to measure it had to be expressed numerically so that comparisons could be made among plants and among populations. The system by which it was obtained had to make possible accurate and rapid examination of large numbers of plants.

Two methods had been used previously. Some workers cut the culms at designated points and described the cross sections as solid, hollow, or intermediate (1, 2, 5, 11, 15, 16, 41, 51, 52). Platt *et al.* (22) split the culm and gave each an index number ranging from 0 for a hollow stem to 5 for a solid stem. Putnam (27) also split the culms but classified them only as solid or hollow.

Cursory examination of the culms of S-615 showed that the top internode (I) tended to have a cavity near the top, to be solid in the center, and sometimes to develop a cavity near the base. In Chinese Spring the top internode had the thickest lining of pith at the top and was thin-walled, hollow at the base. The lower internodes of S-615 were fairly solid and varied less than the top one. The lower internodes of Chinese Spring were hollow. It was important to record not only a total solid stem index but also the pith pattern. For this purpose a modification of Yamashita's method of cross sections (52) and Platt's indices (21, 22) seemed most appropriate.

Three cuts were made with scissors in the top internode (I), at the center, 5 cm below the spike, and 5 cm above the base, and one cut in the center of each of the lower internodes (II, III, IV, and V). After preliminary practice with a ruler, the positions of the cuts were estimated. The solid stem indices were: 1, thin-walled, hollow; 2, thick-walled, hollow; 3, intermediate; 4, very small lumened; 5, solid. The first two of these classes are hollow and the last two essentially solid. The index number 1 rather than 0 was assigned to the most hollow class because even it has a thin lining of pith. Measurements also showed that the sclerenchymatous wall of the stem occupies about 1/6 of the radius and the pith of sections of index 1 about 1/6; each succeeding index number represents the addition of approximately 1/6 of a radius of

pith thickness resulting in index 5 representing 1/6 (sclerenchyma) + 5/6 (pith) = 6/6 of the radius. As assignment of index numbers is by inspection some error is to be expected, but is apparently not great.

The three longest culms of each plant were examined when available and the indices were recorded. The index representing any cut of the plant was found by averaging the indices of the three culms to the nearest whole number, or when only two culms were available, to the nearest half. As most culms had five internodes, this resulted in a total of seven cut indices. When a fifth internode was missing the index of the fourth was used as an estimate of the fifth, and when a sixth was present it was ignored in calculations. This procedure tended to overestimate the index when a fifth was missing and underestimate it when a sixth was present, as the bottom internode was the most solid. The plant's solid total stem index was calculated by adding the individual mean indices of the seven cuts. Thus the maximum solid stem index of a plant was 35, and the minimum 7. This index method makes it possible to treat observations as either enumeration data or measurement data in statistical analyses, provided that there is a measurement concept in the original scale.

Experimental Design

At Swift Current in 1948 and Lethbridge in 1949 and 1950 the tests were sown in single rod row plots with 75 seeds per row. In 1948 and 1949 the plots were completely randomized. As different amounts of seed were available for the various lines, and since it was the original plan to test their fit to a Mendelian ratio, the only purpose of randomization was to eliminate systematic environmental bias. When it became evident that it was desirable to treat solid stem as a quantitative character and test lines by means of analysis of variance, randomized block designs were adopted.

In 1948 all of the 21 chromosomes except IV, XIII, XIV, XVII, XVIII, XIX, and XXI were tested. The normal F_2 and S-615 were included as checks. In 1949 all of the chromosomes except V, VI, VII, XIII, and XIV were tested, with the normal F_2, S-615, and Chinese Spring serving as checks. In 1950 chromosome XIII, the monosomic of which had just been isolated, was tested. The checks included the normal F_2, and the two normal parent varieties. The design was a randomized block with seven replicates. One row of mono-XIII × S-615 F_1 was sown with it.

Because of complications due to the sterility of mono-IV × S-615 F_1, chromosome IV was retested in 1951 in the F_2 hybrid mono-IV × H46145. The latter variety is a solid-stemmed hybrid line that received its solid stem from S-615 and its hybrid with mono-IV is fertile. Checks included the F_2 hybrids of Chinese × S-615 and Chinese × H46145, and varieties S-615, H46145, and Chinese. The test was a randomized block design of single 10-ft row plots, seeded at the rate of 50 seeds per row, with four replicates.

Chromosome XVIII was retested in 1952. F_2 lines tested involved hybrids of mono-XVIII with S-615, H46145, H46146, and Rescue. Their

checks included the corresponding normal F_2 lines, mono-XVIII itself, Chinese Spring, S-615, H46145, H46146, Rescue, and Chinook. The randomized block test consisted of single 10-ft rows sown at the rate of 50 seeds per row, with six replicates. Twenty to 30 plants per row were examined.

In 1953 monosomic XIV, previously unavailable, was tested. Lines from seven different F_1 plants of mono-XIV × S-615 were tested. Checks included the normal F_2, S-615, and Chinese Spring. The randomized block test consisted of six replicates. Each plot was a single 10-ft row sown to 50 seeds. As analysis of previous data showed that for quantitative tests only one culm of each plant need be examined, that method was adopted. Twenty-five plants per row were examined.

Analysis

On the basis of the hypothesis that F_2 hybrids monosomic for chromosomes affecting solid stem would depart from the normal genetic ratio, results for 1948, 1949, and 1950 were treated as enumeration data. As the proportion of solid-stemmed plants could not be determined with certainty, the results were also treated as quantitative data in making comparison among populations. After 1950 only quantitative tests were made.

Two categories were used in the chi-square test of goodness of fit to a genetic ratio, solid and non-solid. Because F_2 variation was continuous no clear-cut categories could be established, so a "best estimate" of the number of solid-stemmed plants was determined empirically. Families that seemed to fit the same ratio were tested for total, pooled, and heterogeneity chi-square (37).

The method of estimating the number of plants in an F_2 population with culms as solid as those of S-615 had to take into consideration the effect of environment on solidness (21, 22). The S-615 plants exhibit a range of solidness with 80% to 90% within a range of three indices, usually centered about an index of 34, but extending occasionally to 28. The F_2 has a frequency distribution with a mean of about 18 to 24 with a mode slightly lower, the numbers of each category diminishing toward the extremes. Therefore, in the F_2 there would be more individuals with a solid stem index of 28 than of 34. Thus, many F_2 individuals within the range of S-615 solidness would not be genetically as solid as S-615, and these could not be distinguished from genetically solid plants less favored by environment. To estimate the number of F_2 plants as solid as S-615, only those were counted that fell within the range of 80% to 90% of the S-615 population, depending on the S-615 distribution. This number was multiplied by the reciprocal of the proportion of S-615 plants within that range to get the corrected number of solid-stemmed plants in the F_2 population.

Analysis of variance of the mean solid stem index of the various populations was performed on all tests. Because 1948 and 1949 results were not designed for this type of analysis much accuracy was lost; the method used was the one for complete randomization as outlined by Cochrane and Cox (3). For all other tests the method was that for multiple classification. For the test of

chromosome XIII in 1950 and XVIII in 1952 separate analyses were done on the solid stem index of the top internode, the second internode, and the combined bottom three internodes.

The F_1 of mono-XIII × S-615 consisted of both monosomic and normal plants. The *t*-test for group comparisons (37) was used to test the difference in their mean solid stem indices.

Association of glume color, awn length, awn hooding, "squarehead", and spike density with solid stem index were tested by both analysis of variance and the *t*-test in both mono-XIII and normal F_2 populations for 1950. Similar tests could not be made in 1948 and 1949 because of wind and rain damage to the plants.

Monosomics of homoeologous group 2, consisting of chromosomes II, XIII, and XX, are morphologically distinctive and associated with solid stem. Solid stem gradients of these lines over several years were graphed to illustrate similarity of the effect of loss of these chromosomes on pith distribution even when, as in mono-II in 1949, comparisons of total solid stem indices were inconclusive.

Experimental Results

Factorial Analysis

Mendelian factorial analysis estimates the smallest number of allelic gene pairs that can account for an observed result. Its success depends on whether phenotypes can be classified accurately with or without further genetic tests such as backcrosses and F_3's. Solid stem segregates in the F_2 in such a way as to produce continuous variation, making it impossible to distinguish clear-cut categories.

Because the proportion of solid-stemmed plants in the normal F_2 grown at Swift Current in 1948 fitted a 27:229 ratio, the product of three dominant and one recessive genes, all families were tested by chi-square for fit to this hypothesis (Table I). F_2 populations involving chromosomes III, VI, VII, XV, XVI, and XX gave satisfactory fits. The total chi-square for these lines and the normal was 6.7082, $df = 7$ and P was between .50 and .30. The pooled chi-square was .0001, $df = 1$, and P was between .99 and .98. The probability for heterogeneity chi-square was between .50 and .30, indicating no interaction. Monosomic lines I, V, VIII, IX, X, XI, and XII fit a 9:247 ratio, and all of these but VIII fit 27:997 ratio, though V did not fit it well. The total chi-square of the fit of monosomics I, V, VIII, IX, X, XI, and XII to a 9:247 ratio gave a probability between .50 and .30, the pooled chi-square gave a P value between .70 and .50, and the heterogeneity chi-square gave a P value between .50 and .30, indicating no interaction. Monosomic VIII fit a 3:61 ratio with a P value between .90 and .80. Mono-II fit a ratio of 81:175 with a P value between .70 and .50. Thus, the best fit for the normal F_2, and monosomics III, VI, VII, XVI, and XX, is 27:229; for monosomics I, V, IX, and XV it is 9:247; and for monosomic II it is 81:175. All of these are 4-factor ratios with dominance changed. The best fit for monosomics X, XI, and XII is 27:997 and for VIII it is 3:61, 5-factor

TABLE I

The number of F_2 plants in crosses of Chinese Spring monosomics by S-615 with stems as solid as those of S-615. Swift Current, 1948

Chromosome tested	Total plants, no.	Solid-stemmed plants (corrected), no.	Test of fit to 27:229 ratio, probability	Test of fit to 9:247 ratio, probability	Test of fit to 27:997 ratio, probability
I	145	6.1*	.02–.01	.90–.80	.50–.30
II	65	18.4††	<.01	—	—
III	54	3.7	.80–.70	.20–.10	.05–.02
V	171	8.6*	.05–.02	.50–.30	.10–.05
VI	266	35.5	.20–.10	<.01	—
VII	263	27.0	.90–.80	<.01	—
VIII	420	20.8**	<.01	.20–.10	<.01
IX	173	7.4**	.01	.70–.50	.30–.20
X	311	7.4**	<.01	.50–.30	.95–.90
XI	105	2.4**	.01	.70–.50	.90–.80
XII	133	2.4**	<.01	.50–.30	.80–.70
XV	67	2.4	.10–.05	.99	.70–.50
XVI	39	3.7	.90–.80	.05–.02	.01
XX	29	2.4	.70–.50	.20–.10	.10–.05
Normal	185	19.6	.98	<.01	—

*Fewer solid-stemmed plants than normal.
†More solid-stemmed plants than normal.

and 3-factor ratios, respectively. On the basis of these data alone it would appear that the loss of chromosomes I, V, IX, and XV of Chinese Spring, and possibly S-615, makes genes for solid stem less effective, and the loss of II makes them more so. The loss of X, XI, and XII caused segregation for one more gene, indicating that these chromosomes may carry recessive genes for solid stem in both parents. Reduction of the number of genes segregating in mono-VIII indicates that chromosome VIII of Chinese Spring may have a gene for solid stem that S-615 does not have. However, because so many of the lines could fit more than one ratio, other tests must be considered in conjunction with this one.

In 1949 at Lethbridge the plants had culms considerably less solid than those grown at Swift Current in 1948. The normal F_2 fit a 9:274 ratio satisfactorily as did monosomic families I, II, III, VIII, IX, X, XI, XVI, and XVII (Table II). For these families the total, pooled, and heterogeneity chi-squares gave P values of .50–.30, .70–.50, and .50–.30 respectively, indicating no interactions. Monosomic lines IV, XII, XV, and XVIII were less solid than normal, but they fit no common ratio. Monosomic lines XIX, XX, and XXI were more solid than normal, XIX and XXI fitting a 27:229 ratio.

The normal F_2 grown at Lethbridge in 1950 fit a 9:247 ratio, but the monosomic XIII line fit a 9:55 ratio. This shows that with the loss of chromosome XIII of Chinese Spring segregation for a gene was eliminated. As the proportion of solid-stemmed plants increased four times, the gene on XIII of Chinese Spring must have been a dominant gene for hollow stem, its allele in S-615 being inactive.

TABLE II

The number of F_2 plants in crosses of Chinese Spring monosomics by S-615 with stems as solid as those of S-615. Lethbridge, 1949 and 1950

Chromosome tested	Total plants, no.	Solid-stemmed plants (corrected), no.	Test of fit to 9:247 ratio, probability	Test of fit to 9:55 ratio, probability	Test of fit to 27:229 ratio, probability
I	242	3.4	.20–.10	—	—
II	349	15.3	.50–.30	—	—
III	315	11.9	.95–.90	—	—
IV	644	0.0**	<.01	—	—
VIII	302	11.9	.80	—	—
IX	257	13.6	.20–.10	—	—
X	87	3.4	.90–.80	—	—
XI	321	6.8	.30–.20	—	—
XII	264	0.0**	<.01	—	—
XV	362	5.1*	.05–.02	—	—
XVI	259	10.2	.90–.80	—	—
XVII	539	22.0	.70–.50	—	—
XVIII	479	6.8**	<.01	—	—
XIX	517	55.9††	<.01	.05–.02	.90–.80
XX	536	37.3††	<.01	<.01	<.01
XXI	494	39.0††	<.01	<.01	.10–.05
Normal	458	17.0	.95–.90	<.01	—
XIII 1950	417	57.5††	<.01	.95–.90	<.01
Normal 1950	485	11.5	.30–.20	<.01	—

*Fewer solid-stemmed plants than normal.
†More solid-stemmed plants than normal.

The ratios observed suggest a minimum of four major gene differences affecting solid stem in S-615 and Chinese Spring. The dominance of genes for pith development is apparently influenced by environment as there were proportionately three times as many solid-stemmed plants in the normal F_2 grown at Swift Current as in the normal F_2 population at Lethbridge in 1949 and 1950. At the same time, loss of certain chromosomes in 1948 appeared to change the dominance relations of the genes by making the lines either less solid as with chromosomes I, V, and VIII to XII, or more solid as in II. Under the less favorable conditions for solid stem in 1949, XII was the only one of this group to have fewer solid-stemmed plants than expected, and loss of II was not effective in increasing the number of solid-stemmed plants.

Monosomics IV and XVIII were more hollow than expected, but they were retested later because the results were probably not valid. Of 644 plants none was as solid-stemmed as S-615. Even if Chinese Spring chromosome IV has a gene for solid stem not present in S-615, there should have been a few solid-stemmed plants in a population of 644. In view of the sterility of the mono-IV × S-615 F_1 it is probable that outcrossing with hollow-stemmed varieties occurred, as spikes were not bagged in the California increase plots. Results of the monosomic XVIII study are questionable because the eight plots that came from two plants had a mean solid stem index of $14.32 \pm .21$,

TABLE III

Mean solid stem index of F_2 hybrids of normal and monosomic lines of Chinese Spring with S-615 and varietal checks. Analysis of variance, complete randomization

Monosomic line or check	Swift Current 1948		Lethbridge 1949	
	No. of plots	Index	No. of plots	Index
I	4	22.4	7	19.8
II	—	—	10	19.2
III	—	—	8	19.6
IV	—	—	19	13.4**
V	4	23.2	—	—
VI	8	24.5	—	—
VII	6	24.0	—	—
VIII	11	21.9	7	18.4
IX	5	22.2	7	18.6
X	9	22.2	3	22.3
XI	3	22.4	7	18.1
XII	4	21.3	7	17.1
XV	—	—	8	19.4
XVI	—	—	7	19.5
XVII	—	—	14	20.0
XVIII	—	—	14	16.1*
XIX	—	—	13	22.5††
XX	—	—	13	21.6††
XXI	—	—	12	21.2†
Normal	4	24.7	14	18.6
Chinese	—	—	7	10.9
S-615	10	34.0	7	33.4
	No significant difference		Significant at 1% level	

*Less solid than normal.
†More solid than normal.

$df = 263$, and the six plots that came from another had a mean of $18.24 \pm .41$, $df = 214$. The normal F_2 had a mean of $18.37 \pm .31$, $df = 457$. As no cytological anomaly was recorded for the F_1, it is probable one of the parents in the cross was segregating for solid stem.

Analysis of Variance

Because of the contradictory results of factorial analysis of F_2 populations, analysis of variance, which takes the solid stem indices of the whole population into account, was employed. Table III summarizes the results of analysis of variance on all families represented by at least three plots in the 1948 and 1949 tests. The F value obtained for the F_2 lines was 1.40 as compared with 2.08, which is the F value at the 5% level with 9 and 48 degrees of freedom. In 1949 the highly significant F value of 26.23 was obtained. Monosomics IV and XVIII were less solid than normal and XIX, XX, and XXI were more solid.

Table IV shows that the total solid stem index of mono-XIII was much higher than that of the normal F_2 population. Analysis of solid stem indices of the top internode, second internode, and the sum of the basal three internodes agreed with that of the total.

TABLE IV

Mean solid stem index of F_2 hybrids of monosomic XIII and normal Chinese Spring wheat with S-615, Lethbridge 1950

Cross or check variety	Mean solid stem index			
	Total stem	Top internode	Second internode	Bottom internodes
Mono XIII × S-615	23.9††	8.60††	2.79††	12.47††
Chinese × S-615	20.3	6.90	2.24	11.14
S-615	33.9	14.09	4.91	15.00
Chinese	12.9	4.16	1.04	7.66
L.S.D. .05	.7	.44	.16	.35
L.S.D. .01	.9	.61	.22	.48

††More solid than normal F_2.

TABLE V

Mean solid stem index of seven F_2 families of monosomic XIV of Chinese Spring × S-615, the F_2 of Chinese Spring × S-615, and their parents, Lethbridge 1953

Variety	Mean solid stem index
(2) 60-2	21.5
(1) 60-5	22.8
(2) 60-4	23.0
(1) 60-6	23.4
(1) 60-2	23.8
(1) 60-4	23.8
(1) 60-3	24.8
Chinese × S-615 F_2	23.2
S-615	34.2
Chinese Spring	12.1
L.S.D. .05	1.8

A summary of results of the test of mono-XIV × S-615 F_2 is given in Table V. The mean solid stem index of the seven monosomic F_2 lines is 23.3 as compared with 23.2 of the normal population and 23.15 as the mean of the parents. Although the least and most solid of the monosomic lines are significantly different, none is significantly more or less solid than the normal check. As a graph of solid stem indices showed pith distribution to be normal in the monosomic lines, no separate analysis was made of the various internodes.

A retest of chromosome IV in 1951 using H46145 instead of S-615 as the solid-stemmed parent (Table VI) showed no significant difference between the normal F_2 population and its monosomic IV counterpart. As it is the effect of Chinese Spring chromosome IV that is being tested, and as H46145 received its genes for solid stem from S-615, these results show that chromosome IV is not critical.

TABLE VI

Mean solid stem index of F_2 and parents, Lethbridge 1951

Variety	Mean solid stem index
Chinese × S-615	20.5
Mono-IV × H46145	19.4
Chinese × H46145	19.1
S-615	31.1
H46145	29.4
Chinese	9.5
L.S.D. .05	1.9

The retest of chromosome XVIII in 1952 using several varieties shows conclusively that loss of chromosome XVIII does not make the F_2 population less solid than the corresponding normal (Table VII). As the normal check in each portion of the test was the progeny of a normal F_1 plant of the hybrid mono-XVIII × variety tested, differences must be attributed to the absence of the Chinese Spring chromosome XVIII only. In every instance the monosomic hybrid was slightly but not significantly more solid than the check. This is due to a more solid top internode, significant in two lines, not to the lower internodes, which are slightly less solid in most of them. S-615 hybrids were less solid than the mean of their parents. The seed source was from the same original cross that provided the seed used in 1949, and again differences

TABLE VII

Mean solid stem index of F_2 hybrids of monosomic XVIII and normal Chinese Spring wheat with solid-stemmed hexaploid, Lethbridge 1952

Cross or check variety	Mean solid stem index			
	Total stem	Top internode	Second internode	Bottom 3 internodes
Mono XVIII × S-615	16.5	6.8††	1.7	8.1
Chinese × S-615	15.7	5.5	1.5	8.7
Mono XVIII × 46145	20.4	8.1	2.3	9.9
Chinese × 46145	20.3	7.8	2.3	10.2
Mono XVIII × 46146	22.8	8.6††	2.6	11.5
Chinese × 46146	21.4	7.3	2.4	11.7
Mono XVIII × Rescue	20.0	7.2	2.1	10.6
Chinese × Rescue	18.8	6.5	2.0	10.4
Mono XVIII	14.8††	5.8†	1.3	7.7**
Chinese Spring	12.3	4.7	1.1	6.4
S-615	31.9	12.4	4.7	14.9
46145	30.7	11.2	4.6	14.8
46146	31.4	11.7	4.8	14.9
Rescue	29.7**	10.5**	4.4*	14.8
Chinook	31.7	12.2	4.7	14.8
L.S.D. .05	1.6	.9	.3	.9
L.S.D. .01	2.1	1.2	.4	1.2

†More solid than normal check.
*Less solid than S-615.

were found among the lines from different plants. As this was also true for the normal check, it must be assumed that discrepancies were due to causes other than the loss of chromosome XVIII. Normal Chinese Spring was less solid than mono-XVIII throughout, significant in total and top and bottom internodes, which makes the results still more conclusive.

Group Comparison of Normal and Monosomic XIII F_1 Plants

As sampling the F_1 plants cytologically would affect their growth, their probable chromosome complement was inferred from that of their offspring. A maximum of four plants was sampled in the progeny of each F_1 plant, and sampling of a line ceased when one monosomic was found. The probability of any F_2 plant from a monosomic F_1 being normal is .25; the probability of four is $(.25)^4 = .0039$. Monosomic plants were found in the progeny of 12 of the 18 F_1 plants. The remaining 6 were probably normal.

The mean solid stem index of the monosomic F_1 plants was 29.58 and that of the normals 26.25. The t value for the mean difference of 3.33 was 3.142, significant at the 1% level for 16 degrees of freedom. The normal F_1 plants had a pair of chromosomes XIII, one from each parent, whereas the monosomics lacked chromosome XIII from Chinese Spring. Therefore, Chinese Spring chromosome XIII must have a gene or genes for hollow stem.

Association of Solid Stem with Other Morphological Characters

An association between solid stem index and any easily recognizable morphological character would provide a clue to the location of genes for solid stem on specific chromosomes. Results of the analysis of variance of solid stem in segments of normal and mono-XIII F_2 populations with contrasting morphological characters are given in Table VIII and the results of the t-test of the same associations are given in Table IX. The test of association of awn length with solid stem index is omitted from Table IX as only pairs of characters were compared by the t-test and four categories of awn length were recorded. Discrepancies in total degrees of freedom for characters recorded in Table IX are due to mutilation of some plants making it impossible to classify for certain characters.

A comparison of Tables VIII and IX shows that the two methods of analysis agree for all characters but glume color. As there was no association in the mono-XIII population by either test and as Platt *et al.* (22) found no correlation between glume color and solid stem, it is likely that the association of white glume with solid stem in the analysis of variance of the normal F_2 population is due to chance sampling.

The association between hooding and solid stem in the monosomic line is the only one that is highly significant. The gene for hooding is on chromosome VIII of Chinese Spring (30, 34). If the association is real, chromosome VIII of Chinese Spring must have a gene for solid stem that S-615 lacks, as shown by the factorial analysis of the 1948 population. In the normal population the genes for solid stem and hooding segregate independently, showing that

TABLE VIII

Analysis of variance of solid stem index in segments of F_2 population having contrasting morphological characters, Lethbridge 1950

Source of variation	Chinese × S-615 F_2			Chinese mono-XIII × S-615 F_2		
	Degrees of freedom	F	Character associated with solid stem	Degrees of freedom	F	Character associated with solid stem
Glume color	1	6.54*	White glume	1	0.376	None
Error	6			6		
Awn length	3	1.01	None	3	4.75*	Short awns
Error	18			18		
Hooding	1	0.141	None	1	16.05**	Hooding
Error	6			6		
Spike density	1	8.71*	Lax spike	1	0.495	None
Error	6			6		
"Squarehead"	1	2.77	None	1	2.46	None
Error	6			6		

*Significant at the 5% level.
**Significant at the 1% level.

TABLE IX

Group comparison of solid stem index in segments of F_2 populations having contrasting morphological characters, Lethbridge 1950

Character	Chinese Spring × S-615 F_2			Chinese Spring mono-XIII × S-615 F_2		
	Mean solid stem index	Degrees of freedom	t	Mean solid stem index	Degrees of freedom	t
Brown glume	20.141	396	1.385	24.064	329	0.678
White glume	21.169	82		23.461	75	
Hooded awns	20.190	342	0.402	24.357	279	2.253*
Straight awns	20.437	141		22.728	135	
Lax spike (2)	20.666	361	2.450*	23.678	295	0.394
Dense spike (3)	19.098	122		23.976	120	
"Squarehead"	19.345	83	1.536	23.424	58	0.458
Tapered head	20.462	397		23.877	357	

*Significant at the 5% level.

they are at least 50 crossover units apart. This association in the monosomic population could be due to decrease in crossing over, as monosomics frequently appear to have reduced metaphase pairing.

The association of short awns with solid stem in the monosomic population is probably another expression of the linkage of a gene for solid stem and the hooding gene, as the hooding gene shortens the awn as well as causing it to curve.

Lax spike is associated with solid stem in the normal F_2 population but not in mono-XIII. Genes for solid stem may be linked with genes for lax spike in S-615. The monosomic plants, constituting about three quarters of the mono-XIII population, have a denser spike than the normals; if chromosome XIII of S-615 also has a gene for hollow stem, which seems unlikely in view of the data, this would tend to counter the expression of association between solid stem and lax spike. Until further evidence is available, this must remain inconclusive.

Pith Pattern in F_2 Hybrids Monosomic for Chromosomes of Homoeologous Group 2

Monosomic F_2 hybrids of chromosomes II, XIII, and XX, which constitute homoelogous group 2 (32, 34), were all more solid than normal in at least one test. Because of the small number of plants examined in the monosomic II population in 1948 and because monosomic II was no more solid than normal in 1949, the effect of chromosome II was in doubt. The mean solid stem index of each point on the stem is plotted in Fig. 1, showing that the pith pattern of monosomic II in 1948 bears the same relation to the normal F_2 as does the pith pattern of the same chromosome to its much less solid normal check in 1949. However, as the spread between them was generally less in the latter, the first cut in the top internode was less solid than normal, resulting in a total solid stem index not significantly higher than that of the normal F_2. Loss of chromosome XX in 1949 made the monosomic considerably more solid, but the pith pattern was essentially the same as that of mono-II.

The pith pattern of mono-XX in 1949 and mono-XIII in 1950 compared with those of their respective normal F_2's is illustrated in Fig. 2. The normal F_2 was more solid in the top internode and less solid in the other internodes in 1949 than in 1950. The pith pattern of mono-XIII bears the same relation to its normal as mono-XX bears to the normal grown in 1949.

Although tests of significance are difficult to apply to these comparisons because data are drawn from three different years in two of which the experiments were not designed for quantitative study, these graphs together with other evidence show that the homoeologous chromosomes II, XIII, and XX affect solid stem in the same way. All have genes for hollow stems. The loss of II makes less difference than the loss of either XIII or XX. Chromosome XIII of Chinese Spring has been clearly shown to have one gene for hollow stem not present in S-615, but no such clear-cut evidence is shown for the other two chromosomes.

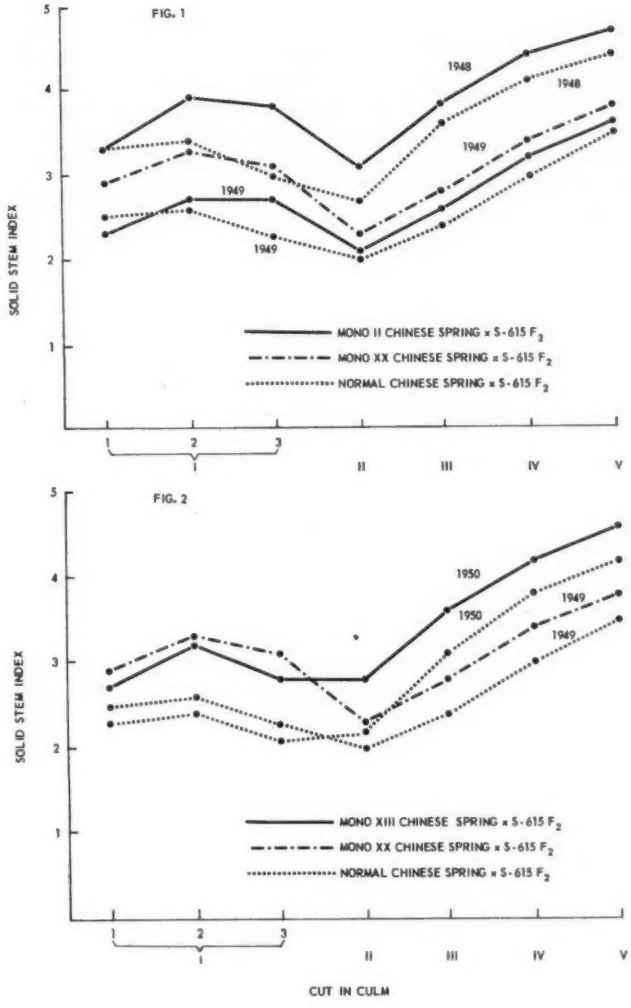

FIG. 1. Mean solid stem indices at various points on the culms of F_2 hybrids involving monosomics II and XX of homoeologous group 2. Roman numerals on the abscissa refer to internodes, numbered from the top. Arabic numerals refer to the three cuts in the top internode.

FIG. 2. Mean solid stem indices at various points on the culms of F_2 hybrids involving monosomics XIII and XX of homoeologous group 2. Roman numerals on the abscissa refer to internodes, numbered from the top. Arabic numerals refer to the three cuts in the top internode.

Discussion

Four monosomic F_2 lines, XIII, XIX, XX, and XXI, were more solid than the normal F_2 population of Chinese Spring × S-615 in every test in which an adequate sample was examined. This shows that these chromosomes, at least in Chinese Spring, carry genes for hollow stem. The change from a tetrahybrid to a trihybrid ratio in mono-XIII shows that Chinese Spring XIII has one gene for hollow stem and S-615 has not. As the loss of the other chromosomes did not change the apparent number of segregating genes, it is probable that in S-615 also they tend to make the culm hollow.

Monosomics I, VIII, IX, X, and XI were less solid than the normal F_2 population in 1948 but not in 1949, when conditions were less favorable for pith development. Generally, this appeared to be due to a change in dominance relations of the genes. Monosomic VIII fits a trihybrid ratio best in 1948, suggesting that Chinese Spring chromosome VIII has a gene for solid stem not possessed by S-615. The linkage between solid stem and hooding supports this view. Mono-XII was less solid than normal in both 1948 and 1949.

There is some evidence that the presence of certain chromosomes affect pith distribution. Although the effect of a loss of the chromosomes of homoeologous group 2 was quantitatively different from chromosome to chromosome and from year to year, the pith pattern of the monosomics had similar relations to their normal checks. Monosomic XVIII, too, in hybrids with several varieties, showed a characteristic pith pattern, tending to be slightly more solid in the top internode and less solid in the lower internodes than the respective normal hybrid.

The only associations of solid stem and morphological characters concerned the hooded awns of Chinese Spring, as mentioned above, and the lax spike of S-615. As spike density is a genetically complex character, the association does not help to locate genes for solid stem.

These results support many previous reports that the D genome tends to make the culm of wheat hollow in that loss of any of the 3 D-genome chromosomes, XIX, XX, and XXI, has resulted in a more nearly solid culm. Chromosome XX carries the gene O_D as identified by Yamashita and Matsumura (17, 18, 53, 54). Of its homoeologues II and XIII, II probably carries the gene m_A in the A genome and XIII has the gene for the hollow stem at locus M_B in the B genome (52).

This F_2 monosomic analysis of a quantitative character has shown that it is far more effective in locating genes in the monosomic parent than those in the variety that is tested against it. No genes in S-615 for solid stem have been located, probably because they are recessive, and the proportion of disomic plants in a monosomic population is approximately one fourth, the same as the proportion of homozygous recessives in a normal F_2 population. Monosomic lines of S-615 should reveal chromosomes critical for genes promoting solid stem.

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STUDIES IN THE GENUS LOTUS (LEGUMINOSAE)

I. LIMITS AND SUBDIVISIONS OF THE GENUS[1]

E. O. CALLEN

Abstract

The genus *Lotus* (Leguminosae) is defined as including *Tetragonolobus* Scop. of the Old World and *Hosackia* Dougl. of N. America, but *Dorycnium* Vill. and *Bonjeania* Reichb. are rejected. There is no single character common to all N. American species which would justify retaining *Hosackia* as a separate genus. Four subgenera are proposed: 1. *Pedrosia* (Lowe) Brand, 2. *Edentolotus* Brand, 3. *Deflectostylus* Callen subgen. nov., and 4. *Tetragonolobus* (Scop.) Callen, using characters of the style (other than size) in separating them. On this basis, approximately half the N. American species fall into subgenus 2, the rest into subgenus 3.

Introduction

The genus *Lotus* (Leguminosae) is world-wide in distribution. In the Old World its main development appears to have centered around the Mediterranean, with one group of species endemic to the western end, and another to the eastern end. A third group has spread down the Nile, through Abyssinia and East Africa to South Africa, whilst a fourth has spread through the Balkans and Asia Minor to Japan. Two species are endemic to Australia, and there is a group of species that extends from the Mediterranean up to the Arctic Circle. In N. America *Lotus* is confined to the Pacific coast, from British Columbia to California and Mexico, and east to Manitoba, Minnesota, and Texas. On the Atlantic seaboard several European species have been introduced and have become naturalized.

Lotus was first described from Europe by Linnaeus (14) and earlier authors. The N. American species were originally described as belonging to *Lotus*, but Bentham (3, 4) placed some of them in *Lotus*, and others in *Hosackia* Dougl. ex Benth. Subsequently Torrey and Gray (29), Gray (9), and Watson (31) placed all N. American species in *Hosackia*. Greene (10) placed them all in *Lotus*, but Taubert (27) and Brand (6) excluded them from *Lotus* and placed them in *Hosackia*. Ottley (19,20,21,22) placed all N. American species in *Lotus*, but Abrams (2) placed them all in *Hosackia* only to have Kearney and Peebles (12) place them all back in *Lotus* again.

It is clear from the foregoing that all European writers (except Bentham) have confined themselves to the Old World *Loti* and have excluded the N. American species from that genus. North American writers have confined themselves to the species of their own continent, but have been quite unable to decide which genus to place them in, and the criteria used by the two groups to distinguish genera, subgenera, and sections have been totally

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different. It is therefore intended in the present work to apply the same yardstick to both groups, with the studies culminating in a biological monograph of the genus *Lotus*.

Limits of the Genus

Although the word *Lotus* has been used since classic times, it was first applied to a trefoil by Camarius in his "Hortus Medicus" of 1588. De Tournefort (28) also used it to describe trefoils, but it was Linnaeus (14) who was the first to define the genus as we know it today.

In the 100 years or so following Linnaeus, many botanists narrowed down the conception of *Lotus* by the creation of other genera into which they placed many of the Linnaean *Lotus* species. Amongst these were: Scopoli (25) with *Tetragonolobus*, Adanson (1) with *Scandalida*, Medicus (17) with *Lotea*, Villars (30) with *Dorycnium*, Muenchhausen (18) with *Krokeria*, Rafinesque (23) with *Lotulus* in 1818 and *Andaca* in 1836, Reichenbach (24) with *Bonjeania*, Lowe (15) with *Pedrosia*, Webb (32) with *Heinekenia*, and Fourreau (7) with *Ortholotus*. Boissier (5) and Jordan (11), by creating further species for *Dorycnium* and *Bonjeania* respectively, also supported the splitting of the Linnaean genus.

After Linnaeus, the first monograph of *Lotus* was that of Seringe (26) in De Candolle's "Prodromus", in which three sections were proposed: *Krokeria* Ser., *Lotea* Ser., and *Eulotus* Ser. The first two of these names were taken from the genera of Muenchhausen (18) and Medicus (17) respectively. *Tetragonolobus* and *Dorycnium* were excluded from *Lotus*. Only two N. American species were known at this time, both placed in *Lotus*. Bentham and Hooker (1865) in their "Genera Plantarum" were the next to monograph *Lotus*, dividing the genus into the following five sections: *Krokeria* Ser., *Lotea* Ser., *Eulotus* Ser., *Microlotus* Benth., and *Ononidium* Boiss. Bentham placed three N. American species into *Microlotus*, leaving the others in *Hosackia*. Greene (10) was of the opinion that all N. American species, as well as *Tetragonolobus* and *Dorycnium*, should be placed in *Lotus*, but he confined his monograph to the N. American species only.

In Engler and Prantl's "Pflanzenfamilien", Taubert (27) revised the genus further. He included *Pedrosia* Lowe and *Heinekenia* Webb as synonyms of *Lotus*, and divided the genus into five sections, *Eulotus* Ser., *Lotea* Ser., *Krokeria* Ser., *Ononidium* Boiss., and *Tetragonolobus* (Scop.) Taubert. However, *Dorycnium*, with *Bonjeania* as a section, as well as *Hosackia* was excluded. Nor did Taubert accept Bentham's *Microlotus*, which he divided into two, placing the N. American species in *Hosackia* and the rest into the section *Eulotus*.

The next and most recent monograph of *Lotus* was written by Brand (6) for Engler's "Botanische Jahrbücher". In it he created two subgenera, *Pedrosia* (Lowe) Brand and *Edentolotus* Brand. For the first of these he proposed two sections: *Heinekenia* (Webb) Brand and *Eupedrosia* Brand, and for *Edentolotus* he proposed five sections: *Krokeria* Muench. (note the

change of authority), *Xantholotus* Brand, *Erythrolotus* Brand, *Ononidium* Boiss., and *Quadrifolium* Brand. He excluded *Tetragonolobus*, *Hosackia*, *Dorycnium*, and *Bonjeania* from *Lotus*, giving as his chief reason the conviction that the genus was large enough already, and that there was no cause for extending it without very compelling reasons (p. 169). He refused to accept Bentham and Hooker's section *Microlotus* on the grounds that it contained a number of N. American species.

The only other major review of *Lotus* appears to be that of Kuprianova (13) in the "Flora of the USSR", in which she has divided the species growing within the Soviet Union into three sections, *Lotea* Ser., *Strictella* Kupr., and *Eulotus* Ser., with a single species, *L. strictus* Fisch. et Mey. in *Strictella*. *Tetragonolobus* is excluded from *Lotus*.

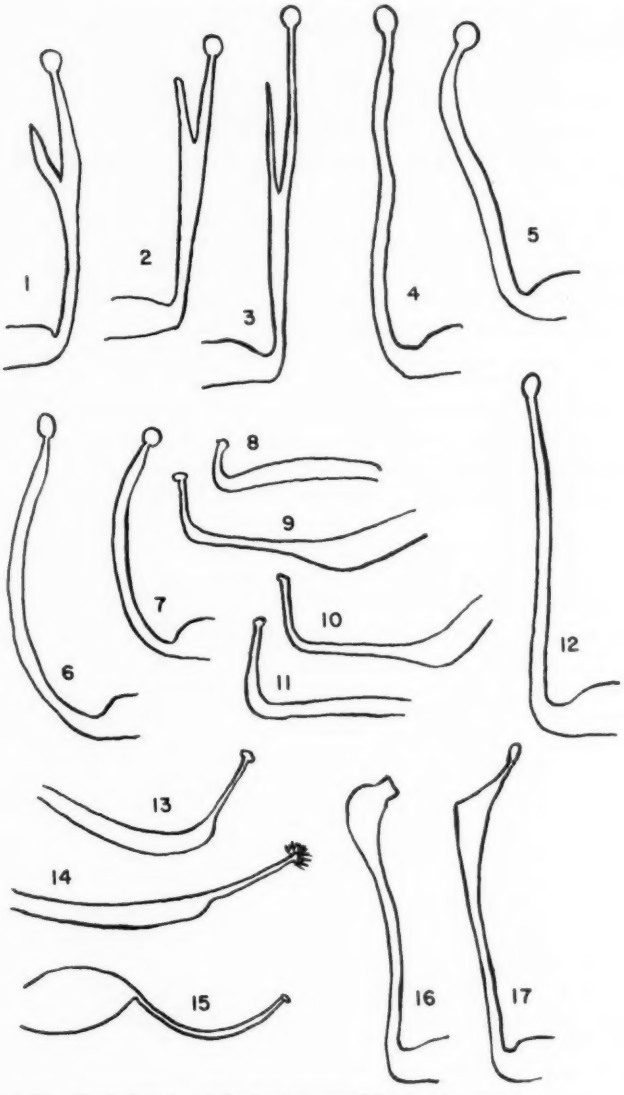
It is clear from this review that the actual limits of *Lotus* are still uncertain. Linnaeus and Taubert included under *Lotus* species separated by all other monographers into *Tetragonolobus*. *Hosackia* is rejected by all monographers of the Old World *Loti*, as well as *Dorycnium* and *Bonjeania*. The present writer chooses to include *Tetragonolobus* and *Hosackia* in *Lotus*, but excludes *Dorycnium* and *Bonjeania*. The reasons for this follow.

The Genus *Lotus*

Brand (6) emphasized three characters as distinguishing *Lotus* from such closely related genera as *Hosackia* and *Tetragonolobus*. They are: (1) leaves possessing five leaflets (except in two species) arranged in a special manner; (2) pods dehiscent; and (3) the absence of foliar stipules. Taubert (27), on the other hand, had emphasized for *Lotus*: (1) the distinctly two-valved, dehiscent pod; (2) the short tubular calyx; (3) the shortly clawed petals; and (4) the distinctly beaked keel, blunt or indistinctly beaked in *Hosackia*. Both, therefore, used the pod as one of their criteria, but for the others, Brand used vegetative and Taubert floral ones. It is clear from a quick perusal of Brand's monograph that the pod alone does not represent a diagnostic character, nor is there any other single character that can be used, but rather a group of characters, both vegetative and floral.

The question of the presence or absence of stipules at one time formed a major point of dispute, of which the arguments for and against have been admirably summarized by MacDonald (16). It is now generally accepted that the Old World *Loti* possess a leaf composed of five leaflets, arranged as two pinnate pairs and a single terminal leaflet. The stipules are reduced to reddish-brown glandular structures on the stem, just above the first pair of leaflets. In the seedling plant these stipules can be seen much more clearly.

In *Tetragonolobus* the stipules are foliar, and the number of leaflets three. As Brand himself pointed out, there is at least one exception to the rule of Old World *Loti* having five leaflets. There is no reason, therefore, for *Tetragonolobus* to be excluded on account of its three leaflets, and *Hosackia* with its three to five leaflets. The pod in *Tetragonolobus* is almost square in cross section, and it has lobes of tissue running the length of the four outside ridges



FIGS. 1–17. Typical styles of the four proposed *Lotus* subgenera.
FIGS. 1–3. Subgenus 1: *Pedrosia* (Lowe) Brand. 1. *L. jacobaeus* L. 2. *L. maroccanus* Lowe. 3. *L. campylocladus* Webb.
FIGS. 4–12. Subgenus 2: *Edentolotus* Brand. 4. *L. tenuis* Waldst. & Kit. 5. *L. ornithopodioides* L. 6. *L. australis* Andr. 7. *L. arabicus* L. 8. *L. incanus* Greene. 9. *L. scoparius* Ottley. 10. *L. argophyllus* Greene. 11. *L. pinnatus* Hook. 12. *L. corniculatus* L.
FIGS. 13–15. Subgenus 3: *Deflectostylus* Callen. 13. *L. strigosus* Greene. 14. *L. grandiflorus* (Benth.) Greene. 15. *L. denticulatus* Greene.
FIGS. 16–17. Subgenus 4: *Tetragonolobus* (Scop.) Callen. 16. *L. tetragonolobus* L. 17. *L. maritimus* L.

of the pod (hence the name). However, as Brand accepted the very curved, fleshy pods of *Lotus edulis* L., the curved, laterally compressed pods of *L. ornithopodioides* L., and the coiled, very thin pods of *L. coimbrensis* Willd., there is no reason why the straight, oblong, winged pods of *Tetragonolobus* should not be included in *Lotus*.

In *Hosackia* about one third of the species have foliar or membranous stipules, the rest having glandular stipules. There is no question of the two types representing separate genera. It would therefore be logical to have the two types represented amongst the Old World *Loti*. This is what, in fact, is being proposed here—with *Tetragonolobus* included amongst the Old World *Loti*, and representing the foliar stipule type.

The number of leaflets borne by the leaves in *Hosackia* varies commonly from 3 to about 11, but in one species (*L. purshianus* Clements and Clements, *L. americanus* Bisch.) towards the tips of the branches, they may be reduced to a single leaflet per leaf, and in *L. stipularis* Greene there may be up to 19.

Taubert specifically mentioned the short, blunt or indistinctly beaked keel in *Hosackia*, which separated that genus from *Lotus*. Examination of the gynoecium enclosed in the keel in the Old World *Loti* shows that the long style is bent at right angles to the ovary (Fig. 12), and that the tip of the keel is drawn out into the form of a beak in order to cover the stigma. In many *Hosackia* species the style is much shorter (Fig. 8), but is also bent at right angles to the ovary. The beak of the keel is therefore shorter and blunter. In other *Hosackia* species the style is short, but not at right angles to the ovary (Fig. 14), and so the keel is not drawn upwards, but drawn forward, and is referred to as being indistinctly beaked. It is therefore not a question of beaks, but rather of the relative length of the style. Actually, within the Old World *Loti* there is quite a variation in the relative length of the style, e.g., Figs. 7 and 12, as also amongst the *Hosackia* species, e.g., Figs. 8 and 11.

On these grounds it is concluded, therefore, that there are no justifiable reasons on which to separate *Tetragonolobus* and *Hosackia* from *Lotus*, and that they must all become members of the genus *Lotus*, as proposed by Taubert (27) for *Tetragonolobus*, and Greene (10), Ottley (19, 20, 21, 22), and Kearney and Peebles (12) for *Hosackia*.

Lotus: calyx short, ± half the length of the corolla, campanulate or bilabiate, calyx teeth subequal; leaves pinnate, with from 3 to 19 leaflets with entire margins; stipules foliar, membranous, or reduced to reddish-brown glands on the stem; flowers single, or in umbels of up to 13 flowers, subtended by 0–3 leaf-like bracts; flowers yellow, pink, white, or varying shades of reddish brown; standard obovate (suborbiculate); keel ± distinctly beaked, keel halves ± joined; stamens diadelphous, 9+1 (five long, filament swollen at tip, other five shorter, alternating, not swollen); pods straight or ± curved, longer than the calyx teeth, oval or square in diameter, usually ± laterally compressed, dehiscent, glabrous or hairy, ± divided internally; (1)3–30(68) seeds.

Subdivisions of the Genus

Taubert (27) separated *Lotus* from *Hosackia*, and in *Lotus* he based his division into sections on three characters: (1) the number of leaflets (3 or 5); (2) the shape of the calyx (campanulate or bilabiate); and (3) the shape or type of pod. Brand (6) also separated *Lotus* from *Hosackia*, and divided *Lotus* into two subgenera, *Pedrosia* (Lowe) Brand and *Edentolotus* Brand, using the character of a toothed style to separate them. Into *Pedrosia* he placed 17 species in two sections, one of these being monotypic. The other 43 species he placed in *Edentolotus*, dividing it into five sections based on: (1) the number of leaflets (3, 4, or 5); (2) the pod; and (3) the color of the flower. Kuprianova (13) used the same names as Taubert for two of her sections, and proposed a new monotypic section *Strictella*, based on differences in the calyx, flower, and leaflets. She did not use the pod to distinguish among sections, as Taubert did.

Brand was the first to divide the genus into subgenera, separating off the species with a toothed or forked style as the subgenus *Pedrosia* (Figs. 1 to 3). These species have a very definite distribution, being restricted to the coastal regions of southwest Spain, western Morocco, and the islands off the west coast of N. Africa, some even being restricted to individual islands. They form a distinct group within the genus, justifying their separation as a subgenus.

Taubert included a section *Tetragonolobus* in *Lotus*, but this was rejected by Brand. An examination of the style shows that there is a very distinct swelling at or near the apex of the style (Figs. 16 and 17), giving it the appearance of being club-shaped, which does not appear in any of the species included in the subgenus *Edentolotus* (Figs. 4 to 12). The stipules are foliar, not glandular, as in *Edentolotus*. In distribution, all but one species are found around the Mediterranean. The exception is *Lotus siliquosus* L. which occurs in eastern, central, and western Europe, the Caucasus, Asia Minor, and N. Africa. Despite its distinctive winged pods, *Tetragonolobus* is not sufficiently distinct from *Lotus* to be a separate genus, but it certainly merits rank as a subgenus of *Lotus*.

Turning to the *Loti* of N. America, we find that the earliest species were referred to the Old World genus (*L. sericeus* Pursh (1814); *L. subpinnatus* Lag. (1816); *L. pinnatus* Hook. (1828)) and that the name *Hosackia* was created only in 1829. In 1837 Bentham still retained 5 species in *Lotus*, and referred the 11 others to *Hosackia*. Bentham and Hooker (1865) retained 3 species in *Lotus*, and placed all other 25 species in *Hosackia*.

On the other hand, Torrey and Gray (29), Gray (9), and Watson (31) referred all N. American species to *Hosackia*, though they differed in the number of sections they accepted for the genus. Bentham (4) created *Microlotus* (in the Old World genus *Lotus*) to accommodate N. American species with single-flowered peduncles and exstipulate leaves. Torrey and Gray (29), on the other hand, placed the section *Microlotus* into *Hosackia*, changing the diagnosis of the section. They also proposed three other

sections: *Euhosackia* Benth., *Syrmatium* Vogel, and *Psychopsis* Nutt. Gray (9) reduced the five species in the section *Psychopsis* to a single one, *Hosackia purshiana* Benth., and placed it in *Microlotus*. He thus accepted three sections to the genus: *Euhosackia* Benth., *Microlotus* Benth., and *Syrmatium* Vogel. Watson (31) followed Gray in using the same three sections.

Greene (10) rejected *Microlotus* on the grounds that Gray and Watson had changed the diagnosis from that of Bentham, and he proposed four sections corresponding to the generic diagnosis of earlier authors: *Acmispon* Raf., *Ansilotus* Bernh., *Hosackia* Benth. (non Dougl.), and *Syrmatium* Vogel. Taubert (27) followed Gray, proposing *Euhosackia*, *Microlotus*, and *Syrmatium*, and based his separation on the character of the pod and shape of the keel.

Ottley was the next to deal with the N. American *Loti*, in Jepson's "Manual of the Flowering Plants of California" (19, 22) in which she proposed the same division as Greene. However, she raised the four sections to subgeneric rank, and based their separation on the characters of stipule, flower number and size, and pod shape and dehiscence. Later (20) she proposed a different subdivision into subgenera, based on characters of stipule, pod, and to some extent the style. These subgenera were: *Hosackia* Benth. (non Dougl.), *Acmispon* Raf., and *Syrmatium* Vogel. This division was later amplified (21) by the creation of a further section to the subgenus *Acmispon*. Abrams (2) proposed the same four subgenera as Greene and Ottley in Jepson's "Manual", except that the first one was called *Euhosackia* Benth. instead of *Hosackia* Benth. He also followed Greene in placing a single species into the subgenus *Acmispon*, namely *Hosackia americana* (Nutt.) Piper (=*H. purshiana* Benth.).

Ottley (21) stated that a careful examination of the N. American species shows that they do not form a distinct group separable from the Old World genus by any one character. It is therefore clear that they do not represent a single subgenus. On the other hand, are there four subgenera as Ottley and Abrams have claimed? The Old World *Loti* can definitely be separated into subgenera on the character of the style. Examination of the N. American species shows that in Ottley's subgenus *Hosackia* all species have a uniform type of style, as have also all but two species in *Syrmatium* (Figs. 8 to 11). Both of these groups have an erect style more or less at right angles to the ovary. In every respect except size, they are identical with the styles of the subgenus *Edentolotus* (Figs. 4 to 12). It is, therefore, proposed that these two subgenera in Ottley's classification be merged with *Edentolotus*, and again become the sections *Euhosackia* and *Syrmatium*.

Ottley's subgenus *Acmispon* Raf. falls naturally into two distinct types on the character of style and stigma. This she utilized in creating the sections *Simpetraea* Ottley and *Microlotus* Benth. However, both have the style attached to the ovary at a very obtuse angle to the vertical (Figs. 13 to 15), which does not occur in any other subgenus of *Lotus*. As the name *Acmispon* has been used by Greene and Abrams for a section or subgenus with but a single species, and as *Microlotus* has been used in different ways by Bentham and Torrey and Gray, neither name is suitable for this new subgenus.

The name *Ansilotus* Bernh. used by both Greene and Abrams is not suitable either, as it definitely excluded the species placed under *Acmispon*, and therefore a new name is required to cover both. As the character on which this new subgenus is being separated from the others is the obtuse angle of attachment of the style to the ovary, I wish to name it: *Deflectostylus* subgen. nov. Type species: *L. strigosus* Greene.

The following subdivisions of *Lotus* are therefore proposed:

Subgenus 1: *Pedrosia* (Lowe) Brand
Style erect, forked or toothed.

Subgenus 2: *Edentolotus* Brand
Style erect, simple.
Incl. sections *Euhosackia* Benth.
Syrmatium Vogel

Subgenus 3: *Deflectostylus* Callen
Style deflected from vertical at obtuse angle.
Incl. section *Acmispon* Raf. s. str.

Subgenus 4: *Tetragonolobus* (Scop.) Callen
Style erect, club-shaped terminally or subterminally.

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NOTES

A SIMPLE METHOD FOR MEASURING THE AREA OF LEAVES OF POTTED PLANTS[1]

ROGER PAQUIN[2] AND LOUIS J. COULOMBE[2]

For measuring leaf area the use of a planimeter appears to be the most accurate method (1). Photographic prints of detached leaves are easily made in a printing box and give a definite outline which is readily followed with a planimeter. However, the method must be modified if surfaces of nondetached leaves are to be measured. To our knowledge, no such modification has yet been reported.

When leaves of a potted plant are to be measured, the plant is held against the edge of a table in a slightly inclined position, the leaf to be printed lying on a circline illuminator (made by General Electric Company). A drawing table with underlights may also be adapted for that purpose. The leaflets are spread out to avoid overlapping and then covered with a sheet of photographic paper and two pieces of plywood ($3/4 \times 6 \times 12$ in.) held together side by side with hinges allowing a 2-cm space between the two for the midrib of the leaf. The pieces of plywood are glued to a single piece of foam rubber or a synthetic sponge. A thin photographic paper such as AD-Type A-1 was found very satisfactory as it reproduces sharply the shape of the leaves. A better contact is obtained by wetting the foam rubber or by interposing a sheet of wet paper between the rubber and the photographic paper. The use of foam rubber prevents damage to leaflets when a uniform hand pressure is applied to the plywood during printing.

The photographic prints thus obtained are as good as those made of detached leaves in a printing box and the outline may readily be followed with a planimeter.

1. WINTER, E. J., SALTER, D. J., STANHILL, G., and BLERSDALE, J. K. A. The growth of leaves. *Edited by* F. L. Milthorpe. Butterworth Scientific Publications, London. 1956.

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NOTES TO CONTRIBUTORS

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